CANCER RESEARCH

VOLUME 11 APRIL 1951 NUMBER 4

SCIENTIFIC PROCEEDINGS

AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

CLEVELAND, OHIO

APRIL 27–29, 1951

[*Authors who are not members of the AACR are indicated by asterisk* (*)]

CYTOCHEMICAL OBSERVATIONS OF CELLS IN HODGKIN'S DISEASE. G. ADOLPH ACKERMAN,* RALPH A. KNOUFF,* and HERMAN A. HOSTER. (Division of Cancer Research, Departments of Anatomy and Medicine, The Ohio State University College of Medicine, Columbus, Ohio.)

Correlated cytochemical and morphologic studies of tissue sections reveal differences between the reticulum cell of "normal" lymph nodes and that of Hodgkin's disease. Cytochemically, Hodgkin's cells contain more sudanophilic and PAS-positive granulation, more ribonucleoprotein, and have a higher acid and alkaline phosphatase activity than normal elongated and round reticulum cells.

Transitional forms between elongated reticulum cells comprising the stroma of Hodgkin's lymph nodes and fully developed Hodgkin's cells and their degenerative forms have been observed. Early evidence of transition is a slight enlargement of the elongated cell and its nucleoli and an increase in acid phosphatase activity. As the transition continues, the cell becomes round, enlarges, and gradually increases in basophilia, sudanophilia, PAS-positive granulation, and acid and alkaline phosphatase content. Sudanophilic and PAS-positive granules are concentrated about the cytocentrum. The nucleus becomes indented and lobulated, the chromatin thickens, and the nucleoli become larger. The toluidine blue-stained cytocentrum, more prominent in Hodgkin's than in normal cells, is identified by a blue area surrounded by lavender cytoplasm. Glycogen and neutral fat droplets are occasionally found in Hodgkin's cells but are not observed in normal round reticulum cells.

The first indication of degeneration of the Hodgkin's cell is a thickening of the nuclear chromatin and an increase in depth of staining and wrinkling of the nucleus. As degeneration continues, cytoplasmic basophilia (toluidine blue) changes from a lavender to a muddy homogenous blue, cytoplasmic sudanophilic and PAS-positive granulation increases, and nuclear and cytoplasmic alkaline and acid phosphatase activity increases.

The cytochemistry of the Hodgkin's cell will be discussed in relation to other lymphoblastomas.

THE EFFECT OF PERIPHERAL HYPOTENSION ON CIRCULATION IN TRANSPLANTED SARCOMAS IN MICE. GLENN H. ALGIRE and FRANCES Y. LEGALLAIS.* (National Cancer Institute, Bethesda, Md.)

The transparent chamber technic was used in making *in vivo* microscopic observations of changes in the blood supply of transplanted sarcomas during experimental alterations in the peripheral blood pressure. Histamine was used to decrease and adrenalin to increase the blood pressure. Arterial systolic pressure was measured in unanesthetized mice by a method described previously (*Fed. Proc.*, 8:349, 1949).

Observations on blood flow in sarcomas and surrounding normal tissues were supplemented by cinematographic records and by measurements of the percentage of tissue area occupied by functional blood vessels.

Direct correlations have been found between the blood pressure decrease in the host peripheral

circulation and the reduction of circulation in tumor capillaries and in capillaries of surrounding tissues of the host. The reduction of tumor circulation is correlated with the duration and degree of the peripheral hypotension.

These results are discussed in relation to the possible role of this mechanism in tumor damage induced by certain chemical agents.

EXPERIMENTAL AND CLINICAL STUDIES ON FLAVONOIDS IN MALIGNANCY. ISIDORE ARONS, JOHN FREEMAN,* and ABRAHAM OPPENHEIM.* (Department of Radiotherapy, Harlem City Hospital, New York, N.Y.)

Sokoloff *et al.* found that a flavonoid compound ($C_{36}H_{30}O_{11}$) inhibits the effect of leukotaxine, an inflammatory factor, on capillary permeability. In their more recent publication they reported that this compound, administered to rats for 30 days, 5 days prior to the exposure and 25 days post radiation, gave considerable protection against a total-body, near-lethal dose of x-radiation. The mortality rate was reduced to 10 per cent, as against 80 per cent in the control. They interpreted their findings as the result of the protective action of flavonoids against capillary injury. In our investigation, rat bearers of carcinoma 175-G were given simultaneously bacterial polysaccharide, 0.5 mg/100 gm wt, and the flavonoid compound, 9 mg/100 gm wt. There was a considerable inhibition of the tumor-breaking effect of bacterial polysaccharide. While the control rats died within 7–9 hours, depending on the size of the tumor, the animals receiving the flavonoid compound remained alive for 68 or more hours. The injury to the adrenal gland, strongly pronounced in the control group, was reduced to insignificant changes in the reticularis. Larger and repeated doses of the flavonoid compound decreased further the toxic reaction of bacterial polysaccharide, with the result that a large proportion of animals so treated remained alive (60 per cent). When investigated clinically (42 cases of cancer of the breast, of the liver, of the rectum, sarcoma of the lungs, carcinoma of the cervix), the flavonoid compound given simultaneously with deep radiation therapy prevented the appearance of radiation erythema of the skin, and patients showed a greater tolerance to x-ray therapy.

AN EVALUATION OF SOME CARCINOGENS AS MUTAGENS. R. W. BARRATT* and E. L. TATUM* (introduced by A. C. Griffin). (Department of Biological Sciences, Stanford University, San Francisco, Calif.)

By use of the fungus *Neurospora crassa* the following carcinogens were tested in the indicated concentrations for mutagenic activity: 20-methylcholanthrene (1/4,000), 9,10-dimethyl-1,2-benzanthracene (1/4,000), 1,2,5,6-dibenzanthracene (1/4,000), *m'*-methyl-*p*-dimethylaminoazobenzene (1/2,000), 4'-amino-2,3-azotoluene (1/1,000), 4-dimethylaminostilbene (1/4,000), and acetylaminofluorene (1/1,000). Microconidia from a homocaryotic strain were inoculated into flasks of nutrient media containing the carcinogen dissolved in Tween 80. After growth for 1 week the culture was transferred to a pectic plate; the resulting microconidia were plated, and cultures derived from isolated colonies were examined for biochemical and morphological mutants. Known mixtures of biochemical mutants, morphological mutants and wild type treated in the above manner showed no selection for the mutant types, under these conditions. The carcinogenic hydrocarbons methylcholanthrene (9,107 cultures examined), 9,10-dimethyl-1,2-benzanthracene (2,501), and 1,2,5,6-dibenzanthracene (1,500) increase the mutation frequency approximately fourfold over the controls (12,431); the azo dyes (6,328) increase the mutation frequency about threefold over the spontaneous frequency; dimethylaminostilbene (2,771) and acetylaminofluorene (2,775) are either weaker mutagens or inactive. The results show that even the most active mutagenic carcinogens are much less active for Neurospora than are nitrogen mustard and radiation. A direct comparison between the mutagenic activity of the two noncarcinogenic hydrocarbons, 1,2-benzanthracene and pyrene, and that of methylcholanthrene is presented, as well as reversion data on several of the biochemical mutants obtained from the carcinogen treatments.

A CHANGE IN THE GROWTH POTENTIALITIES OF A TUMOR AFTER GROWTH IN A HYBRID HOST. MORRIS K. BARRETT and MARGARET K. DERINGER.* (National Cancer Institute, Bethesda, Md.)

A mammary adenocarcinoma which arose spontaneously in a strain C3H female is transplantable to all C3H mice and to all F_1 hybrids between strains C3H and C but not to strain C mice. It also grows progressively in 8 per cent of backcross-to-resistant animals when the graft is taken from a strain C3H donor. However, if the tumor is grown for one generation in the F_1 hybrids and then passed to backcross-to-resistant hosts, it grows progressively in 30 per cent of the latter.

This result, which can be obtained repeatedly, cannot be explained by the usual genetic rules of

transplantation, and it is not due to an ordinary spontaneous mutation in the tumor, such as has been frequently observed in the past. In the absence of exact knowledge as to the nature of the change which occurs in the tumor it is tentatively called an adaptation.

INHIBITORY EFFECTS OF ADRENAL CORTICAL HORMONES ON THE INCORPORATION OF GLYCINE INTO PROTEIN *IN VIVO*. A. D. Barton* and H. P. Rusch. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The incorporation of glycine into various tissue proteins was studied following intravenous or intraperitoneal injection of glycine-2-C^{14} (2–3 μg/gm body weight) into Rockland strain white mice and C3H mice bearing a transplantable mammary adenocarcinoma generously supplied by Dr. Andervont. Some of the mice received no hormone treatment; some received a moderate dose of aqueous adrenal cortical extract (two 0.5 ml. injections subcutaneously, 60 and 30 minutes before injection of the glycine); and some received a relatively large dose of cortisone acetate suspension (0.25 mg. subcutaneously and 0.25 mg. intraperitoneally 4.5 hours before injection of the glycine). The animals were sacrificed, and samples of liver, small intestine, kidney, muscle, and tumor were taken 10, 30, and 60 minutes after injection of the glycine.

The hormone treatment produced inhibitory effects on the incorporation of glycine into the protein of all the tissues studied except tumor. With the lower hormone dosage the inhibitory effect was marked in the liver and kidney, and moderate in the intestine; with the higher hormone dosage the inhibitory effect was also extensive in the small intestine and muscle.

It appears that the increased secretion of adrenal cortical hormones which accompanies stress produces an inhibitory effect on protein synthesis, particularly in the viscera; with large doses of cortisone the inhibitory effect is extended to peripheral tissue. The possible significance of these results in relation to the gluconeogenic, protein-catabolic and cold-survival effects of adrenal cortical hormones, as well as the clinical responses to massive cortisone therapy, will be discussed.

REDUCING SUBSTANCES AS INDICATORS OF PROTECTION AFFORDED BY CORTISONE AGAINST TUMOR-DAMAGING EFFECTS OF *SERRATIA MARCESCENS* POLYSACCHARIDE. Lyle V. Beck. (Department of Physiology and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.)

The literature indicates that necrosis of a tumor, whether spontaneous or induced, is accompanied by a marked decrease in tumor content of the reducing substances, ascorbic acid and glutathione. In the present work, a marked decrease in tumor content of these reducing substances was found to accompany hemorrhagic necrosis induced in mouse Sarcoma 37 by arsenite, alpha-peltatin, and *Serratia marcescens* polysaccharide preparation P25. The reducing substances were obtained in 6 per cent metaphosphoric acid extracts of pooled samples of tumor tissue, secured 24 hours after injection of the tumor-necrotizing agent. The ascorbic acid and glutathione contents of the tumor tissue samples were estimated from iodine and 2,6-dichlorophenol indophenol titrations of these extracts.

Experiments were performed in which groups of mice bearing Sarcoma 37, implanted 6 days earlier, were given, intravenously, graded doses of the polysaccharide preparation P25. At any one dose level of the polysaccharide, one group of five mice received only P25, while another group of five mice received an intraperitoneal injection of cortisone, 5 μg. per gram, 2 hours before the P25. Comparative amounts of induced tumor damage for the cortisone- and noncortisone-injected mice were estimated by gross and histologic inspection. It was judged that the minimum dose of P25 required to induce marked tumor damage was twice as large for cortisone-treated as for noncortisone-treated mice. The minimum dose of P25 required to induce marked decreases in tumor content of ascorbic acid and of glutathione was found to be at least twice as great for cortisone-injected as for noncortisone-injected mice.

NITROGEN AND MINERAL METABOLISM AND STUDIES ON PROTEIN SYNTHESIS IN MULTIPLE MYELOMA. Judith Bellin* (introduced by Daniel Laszlo). (Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

Seven patients with multiple myeloma were studied under controlled metabolic conditions for an average of 69 days each. In these patients, active osteolysis was demonstrated to be accompanied by relatively high urinary calcium excretions and negative calcium balances. In one patient stilbestrol induced improvement of the calcium and phosphorus balances, and testosterone induced nitrogen retention with but slight improvement in the mineral balances.

In two patients with multiple myeloma, the rate of protein synthesis was measured by the method of Rittenberg and Sprinson, by following the urinary excretion of N^{15} when labeled glycine was administered. In the first patient, who had no Bence-Jones proteinuria, the rate of protein synthesis and the size of the metabolic nitrogen pool were normal. In the second individual, who had a Bence-Jones proteinuria averaging 6 gm. per day, the rate of protein synthesis was significantly increased (162 gm/day, versus 98 gm/day for a normal). From the amount of N^{15} incorporated into the Bence-Jones protein, which was excreted in the urine (as followed by electrophoresis and chemical purification), the amount of Bence-Jones protein synthesized was estimated to be twice the amount excreted. It would therefore appear that the turnover rate of proteins in this patient was $1\frac{1}{2}\times$greater than normal.

EXCRETION AND DISTRIBUTION STUDIES OF N^{15}-LABELED *p*-DIMETHYLAMINOAZOBENZENE. Max Berenbom* and Julius White. (National Cancer Institute, Bethesda, Md.)

Three preparations of *p*-dimethylaminoazobenzene, each of which was labeled with N^{15} in a different position, were administered orally to rats weighing 100–120 gm. Studies were made on the excretion of N^{15} in the urine and feces. The rate of movement of the labeled azo dye through the gastrointestinal tract, as well as the deposition in the liver, was determined. Urinary excretion of the N^{15} was inversely proportional to the rate of absorption from the gastrointestinal tract.

Comparisons were made of the N^{15} excretion in rats receiving an initial dose of labeled *p*-dimethylaminoazobenzene with that of those ingesting a diet containing 0.06 per cent of the dye for various periods of time.

THERAPY OF INOPERABLE VISCERAL AND REGIONAL METASTASES BY INTRA-ARTERIAL CATHETERIZATION IN MAN. Howard R. Bierman, Ralph L. Byron, Jr.,* and Keith H. Kelly.* (Laboratory of Experimental Oncology, National Cancer Institute, National Institutes of Health, Public Health Service, Federal Security Agency, and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

The blood supply of primary and metastatic tumors in man is increased. In some viscera, notably the liver, the metastatic lesions are supplied almost exclusively by arterial blood, while the normal liver parenchyma is supplied from the portal vein. The technic of intra-arterial catheterization of the liver, spleen, stomach, colon, kidneys, and other viscera and regions has permitted the study of vascularity of metastases *in vivo*, utilizing roentgenographic methods with radio-opaque material.

In those cases exhibiting abnormal arteriograms found to be characteristic of neoplasms, nitrogen mustard (0.3–2.5 mg/kg body weight) was administered through the catheter to the lesion. Methods to enhance the arterial blood flow and diffusion through capillaries within tumors will be described. More recently, triethylene melamine has been employed.

Ninety-two patients with far-advanced neoplastic diseases with visceral metastases have been so treated. The clinical and histological effects of such large doses of HN2 will be described. Intra-arterial chemotherapy is a practical adjunct to the surgical and radiological attack on neoplasms.

THE BEHAVIOR OF LEUKEMIC CELLS DURING CONTINUOUS CROSS-TRANSFUSIONS BETWEEN PATIENTS WITH LEUKEMIA AND OTHER NEOPLASTIC DISEASES. Howard R. Bierman, Ralph L. Byron, Jr.,* Keith H. Kelly,* and Fauno Cordes.* (Laboratory of Experimental Oncology, National Cancer Institute, and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

A mechanism for the removal of leukocytes from the circulation resides within the pulmonary circulation. This mechanism has been studied by eleven cross-transfusions in man. The lungs of nonleukemic patients can remove almost all the transfused leukocytes, while the lungs of leukemics who have normal or elevated counts are relatively ineffective in such leukocyte removal. A patient with sub-leukemic leukemia, however, removed transfused leukocytes continuously for 26 hours at the highest rate observed.

These studies have permitted determinations of the intra-vascular life span, rates of cell production, and removal during and after the cross-transfusions.

The pulmonary circulation in nonleukemic man has been found to be capable of controlling the number of circulating leukocytes and probably operates as a normal physiologic mechanism. In contrast, the lung removal mechanism in the leukemic patients investigated here exhibited abnormal function. The hypothesis is postulated that leukemia in man may be a disease characterized

by a disparity between delivery and removal of leukocytes rather than one explained by the currently accepted theory of excessive overproduction of leukocytes. The influence such an hypothesis will have upon the etiology, pathogenesis, therapy, and question of neoplastic status of leukemia and other malignant states will be discussed.

MITOTIC ABNORMALITIES CAUSED BY PURINES AND PURINE NUCLEOSIDES IN NORMAL AND MALIGNANT CELLS IN TISSUE CULTURE.

John J. Biesele, Ruth E. Berger,* and Lester Weiss.* (Cell Growth Section, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Addition of certain purines to the medium caused the following mitotic abnormalities in cultures of both embryonic mouse skin and Crocker mouse sarcoma 180: prolonged metaphase, chromosomal lagging, multipolar spindles, unequal divisions, anaphasic bridging and fragmentation of chromosomes, increased aneuploidy, formation of micronuclei, and failure to complete cytokinesis. Especially active was the selectively sarcoma-damaging agent, 2,6-diaminopurine lactate. The same aberrations were caused by adenine sulfate, but higher concentrations were required than of the diaminopurine. Adenine sulfate and 2,6-diaminopurine lactate were mutually antagonistic in their mitotic effects, their inhibition ratio being 1:1 for Sarcoma 180 cells. Another effective agent was 2-aminopurine. Like the others, this produced in normal cells in tissue culture the mitotic defects commonly described for untreated malignant cells. The suggestion is made that upsets in purine metabolism underlie mitotic derangements in cancers.

Purine nucleosides caused less of such "radiomimetic" damage, but other changes appeared. For instance, certain combinations of nucleosides inhibited anaphasic movement and caused toroid resting nuclei of doubled chromosome number to arise.

INHERITED HORMONAL MECHANISMS AND MAMMARY CANCER IN VIRGIN FEMALE MICE.

John J. Bittner. (Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

The development of spontaneous mammary cancer in mice is usually associated with an inherited susceptibility, hormonal stimulation, and the mammary tumor milk agent. By crossing females of the A strain, with a low incidence in virgin females, the transmission of a hormonal mechanism, termed the inherited hormonal influence, may be determined by a high incidence in the F_1 virgin females. Four strains (Z or C3H, D_2, D_8, and C), all of which have a high incidence in virgins when they possess the milk agent, have been found to transmit the inherited hormonal influence.

Smith and the author found that the inherited hormonal influence may be associated with post-castrational adrenal hyperplasia. This observation has been extended by Huseby and Bittner to include mice of the low susceptible I stock. However, although the NH stock (Kirschbaum's line) develops post-castrational and spontaneous adrenal tumors, it does not transmit a hormonal mechanism that gives a high incidence of mammary cancer in $ANHF_1$ virgins; but $ZNHF_1$ virgins show a high incidence.

The genetics of the inherited hormonal influence could not be definitely determined, because the incidence in mice of several back-cross generations was influenced by the source of the mammary tumor milk agent. Definite segregation was seen in the progeny of individual matings.

The inherited hormonal influence is another causative factor in the genesis of mammary cancer in mice and may be necessary for a high incidence in virgin females. Other inherited hormonal mechanisms may be indicated from these studies.

STROMAL PARTICIPATION IN THE RESPIRATION OF TUMORS AS REVEALED BY TETRAZOLIUM CHLORIDE.

Maurice M. Black, Israel S. Kleiner, and Francis D. Speer.* (Departments of Pathology and Biochemistry, New York Medical College, New York, N.Y.)

Tissue slices of spontaneous and transplantable mammary tumors of mice were incubated with triphenyltetrazolium chloride (TTC). After incubation, the tissues were fixed in formalin, sectioned with a freezing microtome, and examined microscopically. While minimal evidence of TTC reduction was found within the tumor cells, the capillaries and stromal fibroblasts were sharply outlined by virtue of intense intracellular formazan deposits. No such staining was found in the stroma or vasculature of nontumor tissues, including adrenal, liver, kidney, lymph node, normal breast tissue, or granulomata. A similar tendency for the staining of the stromal elements has also been observed in a wide variety of human cancer tissue. This staining is also unique in that it is not inhibited by fluoride, malonate, or azide. The histo-

chemical observations were also confirmed by quantitative determinations of the TTC reduced per milligram of tissue. To date, any stromal staining which we have seen in nontumor tissue has been prevented by these inhibitors.

Many human tumors may also show intracellular formazan deposits in the tumor cells as well as in the stroma. In such cases the TTC reduction is usually unaffected by the aforementioned inhibitors.

It would appear that the tumor cells and their associated stroma constitute a unique biochemical and biological entity. Further evaluation of this phenomenon appears worthy of more intensive investigation.

CONCENTRATIONS OF ASCORBIC ACID IN PLASMA AND WHITE CELLS OF PATIENTS WITH CANCER AND NONCANCEROUS CHRONIC DISEASE.

Oscar Bodansky, Felix Wróblewski,* and Blanch Markardt.* (Memorial Center for Cancer and Allied Diseases, New York, N.Y.)

The mean value, 0.79 mg/100 cc, for the concentrations of plasma ascorbic acid in 23 healthy persons was significantly higher than the mean values, 0.39 mg/100 cc, for 43 patients with noncancerous disease, and 0.48 mg/100 cc for 69 patients with cancer. Similarly, the mean value for concentrations of white cell ascorbic acid in the group of healthy persons, 36.1 mg/100 gm, was significantly higher than the values, 23.8 mg/100 gm, for the group of patients with noncancerous disease, and 27.0 mg. for the group of patients with cancer. There were no statistically significant differences between the values for the groups of cancer patients and patients with noncancerous disease.

On the basis of the demonstration that the white cell ascorbic acid is a valid index of the total body stores of ascorbic acid (Lowry, O. H.; Bessey, O. A.; Brock, M. J.; and Lopez, J. A., *J. Biol. Chem.*, **166**:111, 1946), it was found that about 30–40 per cent of the patients with cancer or noncancerous disease had subnormal body stores of ascorbic acid. The plotting of plasma against white cell ascorbic acid concentrations in patients and normal persons yielded a relationship similar to that obtained by Lowry and his associates during ascorbic acid realimentation of normal men.

TESTICULAR INTERSTITIAL-CELL TUMORS IN HYBRID MICE AND INBRED MICE GIVEN ESTROGENS OR ESTROGENS AND DESICCATED THYROID.

J. Boddaert* and W. U. Gardner. (Yale University School of Medicine, New Haven, Conn.)

A total of 335 male mice was used in the present study; 236 hybrid males were reciprocal A×C3H crosses. All were given estrogens dissolved in sesame oil (0.05 cc. weekly, containing either 16.6, 25, or 50 μg. of estradiol benzoate or estradiol dipropionate, 250 μg. of stilbestrol, pellets of estrone weighing 2 mg., or pellets of stilbestrol-cholesterol containing about 2 mg. of the estrogen). Testicular interstitial-cell tumors occurred in 56 of the mice and most frequently in those animals without the mammary tumor inciter. The high incidence of tumors in the latter group was attributable to prolonged survival. Stilbestrol seemed to be a more effective tumor inciter than the other estrogens.

It has been assumed that the testicular interstitial-cell tumors are due to an increased production by the pituitary glands, in estrogen-treated animals of some strains, of interstitial cell-stimulating hormone. Some evidence exists that hyperthyroidism increases this hormone. On this basis, the testes of hyperthyroid mice (0.2 per cent desiccated thyroid in the diet) and of hyperthyroid estrogen-treated mice were studied. Testicular tumors occurred in 12 of 48 mice from susceptible strains given both substances. They appeared somewhat earlier in the mice so treated.

THE ABSENCE OF AN INHIBITING EFFECT OF CALORIC RESTRICTION ON PAPILLOMA FORMATION.

R. K. Boutwell and H. P. Rusch. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The inhibiting effect of caloric restriction on the rate of tumor formation is well known. No exceptions to this effect have been reported for any neoplasm that has been adequately tested. It is of interest, therefore, to report that the formation of papillomas was not inhibited by caloric restriction.

A single application of a 0.6 per cent benzene solution of 9,10-dimethyl-1,2-benzanthracene was made to the skin of 48 mice fed at a high calorie level and to a similar group restricted in calories. Thereafter, a solution of 1 per cent croton oil was applied to the same area twice each week. A rapid induction of papillomas results from this procedure, but no difference in tumor incidence was noted in the two groups. Thus, at 40 days the incidence of papillomas in 46 high-calorie mice was 34 and in 43 restricted mice it was 31, and at 68 days

the incidence of tumors in the two groups was still the same. On the 74th day, croton oil was discontinued, and the application twice weekly of a 0.3 per cent solution of 3,4-benzpyrene to the same mice was begun. By the 152d day, six carcinomas were observed in the mice on the high calorie diet, while no carcinomas were found in the group on the restricted diet. At 194 days, the incidence of carcinomas in 43 mice of the high-calorie group was 32, and in 40 of the restricted group it was 10. It is clear from this experiment that caloric restriction did not retard the development of papillomas, while the formation of carcinomas was inhibited. Whether these results are due to the predominance of the croton oil effect over the tumor-inhibiting influence of caloric restriction or to some subtle intrinsic difference in the response of the two types of neoplasms is unknown.

TRANSPLANTABLE SARCOMA OF THE SALAMANDER INDUCED BY METHYLCHOLANTHRENE.

CHARLES BREEDIS. (Department of Pathology, University of Pennsylvania, School of Medicine, Philadelphia, Pa.)

It has been reported (Breedis, C., *Cancer Research*, **10**:205–6, 1950) that injection of certain carcinogenic substances, notably coal tar, into the forelimb of adult *Triturus viridescens* often resulted in the development of accessory limbs and limb parts. These growths showed a high degree of tissue organization and could not be successfully transplanted.

Of more than 500 *T. viridescens* receiving carcinogenic substances, only 2 have developed malignant tumors. Both of these were in a group of 34 animals that had received a single injection into the forelimb of packed crystals of methylcholanthrene (1–5 mg.) in olive oil. One of the neoplasms arose 6 months after injection as a tumor mass showing no tissue organization. In the other case, a poorly developed accessory limb first grew from the site of injection after 4 months. One year later a sarcoma arose at the base of the accessory limb. Both neoplasms had the morphology of spindle-cell sarcoma and could be readily transplanted to other *T. viridescens*. One tumor is in the fourth passage. Dried, glycerinated, or filtered tumor material failed to transmit the sarcoma.

These results indicate that at least one substance, methylcholanthrene, carcinogenic for mammals, is also carcinogenic for a cold-blooded vertebrate. The induction of accessory limbs by carcinogenic substances further suggests that some carcinogens are capable of inducing highly organized growth as well as neoplasia.

AN INVESTIGATION OF THE CAPACITY FOR CLEAVAGE AND DIFFERENTIATION IN EMBRYONIC CELLS LACKING "FUNCTIONAL" CHROMOSOMES.

ROBERT BRIGGS, ELIZABETH UFFORD GREEN, and THOMAS J. KING.* (The Institute for Cancer Research and The Lankenau Hospital Research Institute, Philadelphia, Pa.)

Rana pipiens eggs lacking "functional" chromosomes were prepared by inseminating the eggs with x-rayed (65,300–98,000 r) *R. catesbeiana* or *R. pipiens* sperm, following which procedure the egg nucleus was removed with a glass needle. These eggs went through the first few cleavages in a regular, though retarded, fashion. Later cleavages were restricted largely to the animal hemisphere, and at the end of 1 day's development partial blastulae were produced with a variable proportion ($\frac{1}{3}$ to $\frac{3}{4}$) of the animal hemisphere cleaved into small cells. Cleavage ceased after about 33 hours. The partial blastulae survived for periods up to 5 days, but no differentiation occurred.

Cytological studies showed that the heavily irradiated sperm chromatin retained the capacity to form chromosome fragments and was distributed to many cells on one side of the egg during blastula formation. However, the main part of the blastula consisted of chromatin-free cells which possessed asters or amphiasters and cleaved as well as did the chromatin-containing ones.

In order to test their capacity for further development, groups of "achromosomal" cells from day-old partial blastulae were grafted to various sites on normal hosts (stages 10–16). The grafts healed in normally, but after periods ranging from a few hours to 4 days the graft cells began to round up, and grafts were lost. In sections the grafts showed no signs of cell division, differentiation, or of increased survival.

THE DISTRIBUTION OF NUCLEOLYTIC ENZYMES WITHIN THE CALF THYMUS CELL.

K. D. BROWN* and M. LASKOWSKI. (Department of Biochemistry, Marquette University School of Medicine, Milwaukee, Wis.)

The distribution of desoxypentosenucleic acid (DNA) within the calf thymus cell has been well established. The distribution of enzymes capable of hydrolyzing DNA, within the calf thymus cell, is the subject of this study.

Calf's thymus, removed from the animal 1 hour after death, was used. The tissue was separated into nuclei, large granules, and small granules plus soluble material. Several technics of homogenization and cell fractionation were tried. In all procedures the pH was maintained at 7.1.

Preliminary results indicate that in addition to the well known desoxyribonuclease, there is a second factor active at pH 4.5. The latter is probably the same as the enzyme described by Maver (*J. Biol. Chem.*, **181**:853, 1949).

Regardless of the method of preparation, the highest concentration of Maver's enzyme was found in the nuclear fraction. In contrast to this, desoxyribonuclease appeared to be less localized, with a minimum activity in the nuclei fraction. The total nucleolytic activity in all fractions was lower at pH 7.1 than at pH 4.5.

RESPONSES OF MITOTIC CELLS IN LIVER AND CARCINOMA TO CONTINUOUS INTERNAL IRRADIATION. Austin M. Brues and Leola Rietz.* (Division of Biological and Medical Research, Argonne National Laboratory, Chicago, Ill.)

Regenerating rat liver was exposed to internal beta irradiation by insertion of glass beads containing Y^{91} or Ce^{144}-Pr^{144}. The distribution of the absorbed energy around these point sources was such that concentric spheres of the tissue received graded dosages varying between zero and amounts sufficient to cause early necrosis.

The frequency of mitotic figures was reduced more or less uniformly at a given dosage rate between 8 and 48 hours after insertion, and followed an exponential depression with increasing dosage. Mitotic figures decreased in number in the heavily irradiated areas within 1 hour after insertion of the radioactive source, and virtually disappeared between the fourth and eighth hour. A few residual figures probably represent prolongation of a few mitoses under continuous irradiation.

Exposure of the Hall mouse carcinoma under the same conditions resulted in a similar picture. In addition to this, large cells appeared in the region of depressed mitotic activity. It appears probable that this results from continued nuclear and cytoplasmic growth where mitosis is inhibited.

THE SIGNIFICANCE OF TIME-DOSE RELATIONSHIPS IN CARCINOGENESIS. Austin M. Brues and George A. Sacher.* (Division of Biological and Medical Research, Argonne National Laboratory, Chicago, Ill.)

It is generally agreed that carcinogenesis is more than a simple, single-event process. The examination of time-incidence curves obtained over a range of dosages reveals some interesting, otherwise unrecognized characteristics of the carcinogenic process, and sometimes permits a decision as to which of the several large classes of induction mechanisms might be operative. Three broad classes of statistical hypotheses might be characterized as: (*a*) rare-event or hit theory (single or multiple), (*b*) individual variance in susceptibility, and (*c*) random variation in susceptibility. Hypotheses (*b*) and (*c*) imply that susceptibility as a function of time after exposure is related to some variable physiological characteristic, whereas (*a*) in its pure form implies that susceptibility is a function only of the state of the organism at the moment of exposure. The actual carcinogenic process in a mixed population is undoubtedly a mixture of these three models.

From several points of view, radiation carcinogenesis appears to afford the most reproducible means of investigating these problems, since biological variance among species appears to be at a minimum, and since the stimulus is physically well defined and can be controlled easily in terms of time and space.

The problem of distinguishing between these mechanisms by statistical (actuarial) methods is discussed. Pertinent data are presented in the field of radiation carcinogenesis and compared with results of other carcinogenic stimuli.

PREPARATION OF CELL SUSPENSIONS FROM LIVER AND INVESTIGATION OF THE PROPERTIES OF FREE CELLS. Nancy L. R. Bucher, Jesse F. Scott,* and Elizabeth Simpson.* (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston, Mass.)

We have initiated a study of the properties of separated tissue cells functioning apart from their normal adjuvants and supporting structures.

The method of cell separation is based upon the observation of St. Aubin that hepatic cells which appeared intact could be mechanically shaken loose from rat liver slices with glass beads in a suitable suspending medium. Other types of cells and debris may be removed by repeated centrifugation and resuspension. The resultant preparations contain whole, single hepatic cells, a few partially disintegrated cells, a few clumps of 2–10 cells (about 5 per cent), and, rarely, larger clumps. The average yield is approximately 10 per cent of the number originally present.

In addition to the mechanical forces involved, the yield is influenced by the temperature during

the shaking process, and the composition of the suspending medium; low temperatures (0°–4° C.) and the presence of calcium ions (0.001 M) significantly inhibit cell separability.

Manometric studies have been carried out with various substrates to compare the oxygen consumption of separated cells with that of slices and cell-free homogenates, and to determine the effect of the composition of the suspending medium upon the functional capacity of the cells.

Morphological characteristics of cells have been observed by phase contrast and ultraviolet microscopy; those in the non-ionic media differ from those in ionic media, in both appearance and function. These preliminary experiments with washed suspensions of separated cells are intended as an approach to investigating properties of normal and malignant tissues at the cellular level.

FURTHER STUDIES ON THE MECHANISM OF RESISTANCE TO THERAPY IN LEUKEMIA. JOSEPH H. BURCHENAL, R. ALISON ARMSTRONG,* and GEORGIA M. BABCOCK.* (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

The therapy of those cases of acute leukemia responding at first to the folic acid antagonists or the hormones ACTH or cortisone fails, eventually, because of the development of resistance to therapy. An analogous situation has been demonstrated in a strain of mouse leukemia sensitive to A-methopterin. A subline of this leukemia has been made resistant to the drug by passage for several generations through treated mice. This resistance is considered the result of a random mutation, selected out by the presence of A-methopterin. The resistance has remained unchanged for over 30 passages through untreated mice. Studies have been made on the sensitive (Ak4) and the resistant (Ak4R) sublines, in an attempt to elucidate the mechanism of this resistance. Morphologically, the sublines are indistinguishable. The Ak4R line is resistant to all the 4-amino derivatives of PGA tested, but it retains its sensitivity to 2,6-diaminopurine and, when on a low PGA diet, to the weak antagonist of PGA, 9-methyl-PGA.

Although the mechanism of resistance in arsenic resistant trypanosomes has been shown to be a decreased ability to adsorb or absorb the chemotherapeutic agent, this does not seem to be the explanation of Ak4R resistance to A-methopterin. Ak4R cells do not contain significantly more PGA or citrovorum factor than those of the Ak4 line. The most likely mechanism of resistance, at present, appears to be a detoxification of the A-methopterin molecule by some alteration of the 4-amino group or, less probably, the development of an alternate metabolic pathway which bypasses the PGA-CF system.

TUMOR INCIDENCE AND LETHAL MUTATION RATE IN DROSOPHILA FOLLOWING TREATMENT WITH FORMALDEHYDE. WALTER J. BURDETTE. (Department of Surgery, School of Medicine, Louisiana State University, New Orleans, La.)

If tumors are formed as a result of somatic mutation, it should follow that agents which produce mutations also increase tumor incidence, since no chemical or physical agent is known to cause directed or specific mutations. Although it has been shown that certain agents such as x-rays and nitrogen mustard cause both mutation rate and tumor incidence to increase, an instance where mutation rate is increased without a change in the percentage of tumors would suggest that this may be a coincidence, particularly if the two are determined simultaneously.

It is possible to determine lethal mutation rate and tumor incidence at the same time in Drosophila, and flies of the tu 36a strain were tested by this method. They were raised on laboratory medium containing formaldehyde (0.1 per cent), and both these flies and their offspring were examined for tumors. The males were mated to Muller-5 virgin females, and the lethal mutation rate determined. For every experimental culture a parallel control culture was also tested.

In the treated group, fifteen tumors were found among 924 flies (1.62 per cent), whereas in the control group fifteen tumorous flies were found among 861 (1.74 per cent). There is clearly no significant difference in tumor incidence between the two groups ($P = 0.79$). This is also true for tumor incidence in their F_1 progeny. However, there is a difference in lethal mutation rate between the two groups ($P = 0.0001$). Eighteen lethals occurred among 1,174 X chromosomes tested in the treated group, and one lethal mutation appeared among 1,217 chromosomes without treatment.

It is evident that an increase in mutation rate has not resulted in an increase in tumor incidence. Such evidence does not support the hypothesis that tumorigenesis may be explained by somatic mutation.

CLINICAL EVALUATION OF A SERO-FLOCCULATION REACTION IN 300 CASES OF CHEST MALIGNANCIES. F. X. BY-

RON,* GEORGE C. HALL,* HARRY S. PENN, ANDREW H. DOWDY, and A. W. BELLAMY.* (Tumor Control Center, Surgical Service, Wadsworth General Hospital, Veterans Administration Center, and the Department of Radiology and Surgery, University of California, School of Medicine, Los Angeles, Calif.)

This is to report on approximately 300 cases of malignancy involving the chest, with the use of the sero-flocculation reaction as an aid in diagnostic procedure. An arbitrary grading of lesions was used in the breakdown for clinical evaluation. The antigen consisted of the unsaponifiable fraction derived from human cancerous tissue. About half of the sera were tested simultaneously with a fraction synthesized by one of us (H.S.P.) from bile acids. The results obtained in both tests gave above 90 per cent correct diagnoses in biopsy-proved malignancies. Normal controls gave a maximum of 1 per cent presumably false-positive reactions. Infections, such as active tuberculosis, syphilis (gummatous lesions of the mediastinum), gave false-positive reactions in 17–23 per cent of cases studied. Febrile cases, such as pneumonias, gave approximately 6 per cent false-positives. Empyema and lung abscesses showed a relatively negative flocculation reaction in most instances. Some cases in which the diagnosis of empyema or lung abscess was made clinically, and whose sera gave positive reactions, subsequently proved to be early malignancies by histopathological examination.

THE MECHANISM OF CHEMICAL INDUCTION OF VISCERAL TUMORS IN THE SWISS ALBINO MOUSE BY MEANS OF CUTANEOUS ADMINISTRATION OF CARCINOGENS.

PERIHAN CAMBEL and FRANCIS E. RAY. (Cancer Research Laboratory, University of Florida, Gainesville, Fla.)

Methylcholanthrene (MC) was applied in 0.6 per cent benzene solution to the shaved back skin of Swiss mice 3 times in 1 week. In addition to lung adenomas, papillomas and one carcinoma of the forestomach were observed after a very long period of incubation. The incidence of the forestomach tumors was much higher than that of the spontaneous forestomach tumors observed by Maud Slye. No skin or visceral tumors could be induced in the albino rat by standard methods known to induce skin and lung tumors in the Swiss mouse. Therefore, the existence of tissue barriers in the rat skin has been postulated. One of these barriers protects the rat against visceral tumorigenesis by polycyclic hydrocarbon carcinogens. In later experiments, forestomach papillomas, lung adenomas, and thymomas have been observed in Swiss mice after the cutaneous administration of either MC or DMBA. These experiments show the possibility of chemically inducing visceral tumors, especially forestomach tumors, after a long period of incubation by the cutaneous application of subthreshold doses of powerful carcinogens. The experiments also indicate that visceral tumors can be induced upon cutaneous administration of carcinogens only in certain species that have no tissue barrier in the skin preventing the further penetration of carcinogens from the sebaceous glands to the subcutaneous fat. The importance of tissue and blood barriers is discussed in its relation to chemical carcinogenesis.

QUANTITATIVE COMPARISON OF SUBMICROSCOPIC CYTOPLASMIC PARTICULATES OBSERVED IN NORMAL AND MALIGNANT CELLS WITH THE ELECTRON MICROSCOPE.

CECILY M. M. CANNAN* and RUTH E. BERGER* (introduced by Alice E. Moore). (Sloan-Kettering Institute, New York, N.Y.)

Cells from a variety of sources have been grown on formvar films in roller-tube tissue culture and thence transferred to electron-microscope grids for observation according to established methods. Both adult and embryonic mouse fibroblasts and epithelial cells, together with sarcoma and carcinoma cells, have been studied in this manner with respect to structures found in the cytoplasm. The human material examined has been: fibroblasts, epithelial cells, and neoplastic cells grown from more than ten biopsy specimens from Memorial Hospital. All cells, after osmic acid fixation, disclosed only the three types of cytoplasmic particulates already reported: mitochondria, microsomes, and certain osmiophilic rods and granules, with an average diameter of 0.12μ. A comparison has been made of the relative occurrence of these structures in the normal, embryonic and neoplastic cell studied, with particular reference to their significance with respect to the neoplastic process which has been considered by others. These granules are shown to occur in a significantly much greater number in neoplastic and in a slightly greater number in embryonic than in normal cells. Their relation to other optical comparisons of malignant and normal cells will also be discussed. Representative electron micrographs will be shown in slides.

A COMMON PROPERTY OF SEVERAL TUMORS ASSOCIATED WITH A QUALITATIVE CHEMICAL CHANGE IN CARCINOGENESIS.

C. CARRUTHERS and V. SUNTZEFF. (Division of Cancer Research, Washington University School of Medicine, St. Louis, Mo.)

Previous studies have shown that polarographically reducible substances present in epidermis, muscle, and liver of mice differ in their half-wave potentials or in their absorption characteristics in the ultraviolet, yet the reducible substance in the tumors derived from these three tissues has some common properties.

That reducibility at the dropping mercury electrode and absorption in the ultraviolet are common properties of the compounds in the three normal tissues was proved by countercurrent distribution in nine separatory funnels between water saturated with *n*-butyl alcohol against the latter saturated with water. The substance from epidermis concentrated maximally in tubes 6 and 7, with a good correlation between the diffusion current, i_d, and optical density, O.D., measured at 285 mμ. The substance from muscle concentrated in tubes 3 and 4, with a good correlation between i_d, and O.D., measured at 260 mμ at pH 1.5. The substance from normal liver was concentrated in tube 4, with a fair correlation between i_d, and O.D. measured at 260 mμ when both the *n*-butyl alcohol and the water phases were combined, and a good correlation when only the *n*-butyl alcohol layer was used. The substance from the three tumors had a common pattern in that the O.D. was concentrated in tubes 6 and 7, and i_d in tubes 3 and 4. It would thus appear that the alteration in the reducible compound is also associated with its cleavage in malignancy, resulting in a common property of the tumors examined.

STUDIES OF THE ANTI-CATALASE-PRODUCING SUBSTANCES OF MALIGNANT TUMORS. I. PRODUCTS RELATED TO HEMOGLOBIN. THE ACTION OF SANGUININ.

J. K. CLINE, ROBERT B. JOHNSON,* ELIZABETH SCHOLZ,* and CECILE MORGAN WEBB.* (Cancer Research Department, Medical College of Alabama, Birmingham, Ala.)

Lack of knowledge of any substance in the circulating blood specifically related to malignancy has hampered development of suitable laboratory diagnostic tests. Recently, we have undertaken an investigation of those factors related to malignant tumors which apparently lower liver catalase production *in vivo*, namely: (*a*) the factor in malignant tumors which lowers liver catalase in tumor-bearing animals first described by Greenstein (*J. Nat. Cancer Inst.*, **2**:525, 1942), (*b*) the factor extractable from malignant tumor described by Nakahara and Fukuoka (*Gann*, **40**:45, 1949), and (*c*) the factor present in tumor transplants, unrelated to the growth of the tumor, described by Adams (*Brit. J. Cancer*, **4**:183, 1950). These factors may be identical.

Samples of serum from human cancer patients in amounts up to 5 cc. did not contain enough anti-catalase-producing substance, when extracted by Nakahara's method, to lower liver catalase in mice. Very small amounts of tumor transplants lowered liver catalase in male Swiss mice in 24 hours. Sanguinin, a tryptic digest product derived from hemoglobin, reported to have antibiotic activity by Anigstein (*Proc. Soc. Exper. Biol. & Med.*, **74**:346, 1950), was kindly supplied by him to us. It was found to be effective in lowering liver catalase in C57 and C3H mice in a 24-hour period.

Studies of the possible mechanism of action and toxicity and the comparison of the properties of sanguinin with the tumor extracts and tumor transplants will be reported.

EFFECT OF CORTISONE ON GROWTH OF A MIXED-CELL SARCOMA IN THE HAMSTER.

E. D. CRABB and MARGARET A. KELSALL. (Beta Sigma Phi Cancer Research Foundation, University of Colorado, Boulder, Colo.)

Eleven adult males received 3–31 subpannicular injections of cortisone acetate (0.15 mg/100 gm body weight) during 5–67 days before implantation with passages 67 and 68 of Sarcoma Lung Line 6, and 6–15 injections during 8–21 days after implantation. As controls, 8 males were implanted with the same tumor, and 15 others received cortisone only.

Eight to 21 injections of cortisone every other day for 16 days depleted the lymphoid tissue, and reduced the normal circulating lymphocyte count in jugular blood from 60 to 80 per cent (1,350 to 2,696 lymphocytes per cubic millimeter) for 12 normal hamsters to 1–20 per cent (89 to 257) for the 9 cortisone controls receiving 8 or more injections.

In the cortisone-tumor group, the blood and lymphoid picture was quite similar to that in the cortisone control group; but tumor growth was retarded compared to that in the tumor control group, and tumors 6–21 days after implantation were more extensively necrotic.

Periportal infiltration of lymphocytes and

plasmacytes in the liver and hematopoiesis in the spleen were absent in the cortisone tumor group but occurred in the tumor controls, and are characteristic of the tumor line.

PROPERTIES OF CONJUGATES PREPARED FROM PROTEINS AND SYSTEMIC CARCINOGENS. Hugh J. Creech, Richard M. Peck,* and H. Francis Havas.* (The Institute for Cancer Research and the Lankenau Hospital Research Institute, Philadelphia, Pa.)

The synthesis and testing of conjugates prepared from proteins and systemic carcinogens have been undertaken because of the experimental difficulties associated with inhibition of the localized carcinogenic activity of polycyclic aromatic hydrocarbons by prior immunization of animals with hydrocarbon-protein conjugates. Isocyanates of 4-dimethylaminostilbene (I) and 2′-methyl-4-dimethylaminostilbene (II) have been synthesized from the amines prepared from the condensation products of *p*-dimethylaminobenzaldehyde with *p*-nitrotoluene and 4-nitro-*o*-xylene, respectively. These isocyanates have been coupled with horse serum albumin and bovine serum albumin in an aqueous dioxane medium. Determinations by ultraviolet spectrophotometry indicate that the present conjugates prepared from the 4′-isocyanate of I contain about 20 prosthetic groups per molecule of protein, whereas those from the 4′-isocyanate of II contain almost 50 groups. Conjugates containing 2-acetylaminofluorene as prosthetic group are also being prepared.

Injection of the stilbene conjugates into rabbits elicited the formation of satisfactory antisera. Relatively strong prosthetic group activity has been demonstrated by standard precipitin tests and by micro-Kjeldahl analyses. Haptenic specificity was found not to be absolute; for example, antisera toward 4-dimethylaminostilbenyl-4′-carbamido horse serum albumin reacted not only with 4-dimethylaminostilbenyl-4′-carbamido bovine serum albumin but also with 2′-methyl-4-dimethylaminostilbenyl-4′-carbamido bovine serum albumin. Work is in progress to determine the influence of amino acid conjugates of these carcinogens in inhibiting the reactions of the appropriate antisera with homologous carcinogen-heterologous protein antigens and heterologous carcinogen-heterologous protein antigens.

HEMOANTIBODIES IN TUMOR-BEARING MICE. Israel Davidsohn and Kurt Stern. (Department of Pathology, Chicago Medical School, and Mount Sinai Medical Research Foundation, Chicago, Ill.)

Natural agglutinins and immune agglutinins and hemolysins for sheep and chicken erythrocytes were compared in normal and leukemic Akm mice, and in tumor-free and tumor-bearing mice of C57 black and C3H strains. Significantly lower titers of immune hemolysins for sheep and chicken erythrocytes were found in Akm mice with transplanted leukemia, whereas natural and immune agglutinins were not affected by the presence of leukemia. Presence of transplanted and induced tumors in C57 blacks and C3H did not influence the natural agglutinins for sheep and chicken erythrocytes, as found by determining the antibodies before and after development of leukemia or tumors, respectively. Induced or transplanted tumors in C3H and transplanted tumors in C57 blacks failed to influence titers of immune hemoantibodies. Immunization with sheep and chicken erythrocytes did not affect development and growth of leukemia and of tumors; gross anatomic and histologic findings were similar in injected and noninjected animals.

LIVER CATALASE ACTIVITY IN RATS BEARING BENIGN AND MALIGNANT TUMORS. T. E. Dickinson* and R. W. Begg. (Department of Medical Research, University of Western Ontario, London, Canada.)

Liver catalase activity has been determined in rats bearing the benign mammary fibroadenoma described by Millar and Noble and in the sarcomatous derivative. In rats bearing the benign tumor, liver catalase activity is 10 per cent less than in controls, a difference which is not significant. The malignant tumors induce a loss of 50 per cent of liver catalase activity. The benign tumor does produce a significant degree of anemia. These findings will be discussed in relation to tumor growth and malignancy.

A COMPARISON OF THE DISTRIBUTION OF ISOTOPIC NITROGEN AND DIAZOTIZABLE NITROGEN OF N^{15}-2-ACETYLAMINOFLUORENE IN THE RAT. Helen M. Dyer, Helen E. Ross,* and Harold P. Morris. (National Cancer Institute, Bethesda, Md.)

Acetylaminofluorene containing 60 atom per cent excess N^{15} was administered to rats by stomach tube in single and repeated doses. The animals were transferred to metabolism cages for

collection of the excreta. In some cases bile was collected through a bile fistula. After various intervals the animals were killed, and the tissues were analyzed for N^{15} by the mass spectrometer and for diazotizable nitrogen by the method of Westfall (*J. Nat. Cancer Inst.*, **8**:17, 1947).

The results indicate that large portions of the carcinogen, measured as both N^{15} and as diazotizable nitrogen, pass through the bile and that most of the material present in the bile has become more soluble in water than in ether. The results also support the theory that a large portion of the nitrogen of the carcinogen is present in the tissues of the rat in a form that is not detectable by the diazotization method.

DOSE-RESPONSE RELATIONS IN EXPERIMENTAL TRANSMISSION OF AVIAN ERYTHRO-MYELOBLASTIC LEUKOSIS. EDWARD A. ECKERT,* DOROTHY BEARD,* and J. W. BEARD. (Duke University School of Medicine, Durham, N.C.)

Erythro-myeloblastic leukosis is a virus-induced neoplastic disease of chickens, characterized by the appearance of large numbers of primitive cells in the blood stream. Experimental transmission of the disease can be effected by transfer of either filtered plasma or whole blood to susceptible hosts. Transmission in the former instance may be regarded as related to virus activity alone and in the latter to virus and primitive (malignant) cells acting separately or in concert.

Studies have been made of dose-response relationships, for the purpose of developing procedures suitable for bioassay of the various factors concerned with transmission. Analyses of the results obtained with whole blood in 120 3-day-old chicks for each of six dose groups per experiment revealed linear relationships (*a*) between logarithm of dose and reciprocal of latent period and (*b*) between logarithm of dose and incidence of positive inoculations expressed in probits. Analogous experiments with filtered plasma have shown a linear relationship between logarithm of dose and incidence expressed in probits. Qualitatively, the results with whole blood were similar to the respective findings with filtered plasma, differing quantitatively, though, in (*a*) a much shorter latent period, (*b*) a considerably higher incidence of positive inoculations, and (*c*) a much broader effective dose-range. It is evident that the experiments provide a basis of quantitative relations adequate for practical and useful bioassay of the factors of whole blood and of filtered plasma responsible for the induction of this form of leukosis.

THE EFFECT OF NEGATIVE IONIZATION ON TRANSPLANTED TUMORS. W. H. EDDY,* L. STRELTZOV,* J. WILLIAMS,* and L. SCIORTINO* (introduced by B. Sokoloff). (Southern Bio-Research Laboratory, Florida Southern College, Lakeland, Fla.)

E. Kustner and C. Dittmar reported that exposure of tumor-bearing mice and rats to air which was brought to a strong negative ionization delayed the appearance and inhibited the growth of transplanted tumors. A similar inhibitory effect of negative ionization was noted in spontaneous tumors as well as on benzpyrene-induced growth. In the present work, the effect of negative ionization on transplanted rat carcinoma 175-G and mouse adenocarcinoma E 0771 was investigated. The animals were placed in a special cubicle the day of transplantation. The density of negative ions per cubic centimeter of air was maintained at about a 6,000–8,000 level. The animals were kept in these cubicles continuously, except for short intervals required for feeding, for 20–25 days. Considerable inhibition was observed in rat carcinoma 175-G. At the end of 3 weeks, the average size of the tumors in treated animals was 5.2 cc. as compared to 9.9 cc. in controls. At the end of 4 weeks, the average size was 6.5 cc. as against 12.5 cc. A greater inhibition was achieved when the rats kept in the atmosphere of negative ionization were given calcium lactate, 5 mg/100 gm body wt daily. While calcium lactate produced only a slight inhibition (average size 9.9 cc. compared to 10.4 cc. at the end of 4 weeks after transplantation), the average size of tumors of the rats kept under negative ionization and given calcium had remained almost stationary: 2.7 cc. at the end of 4 weeks after the transplantation. An investigation to determine if negative ionization enhances the retention of calcium in malignant tissue is under way.

HOST RESISTANCE TO TUMORS TRANSPLANTED TO EXTRA-OCULAR CRANIAL TISSUES. E. J. EICHWALD and H. Y. CHANG.* (Department of Pathology, University of Utah Medical School, Salt Lake City, Utah.)

Mouse neuroblastoma C1300 will invade and grow in the subcutaneous tissue of the face and neck of C57 brown mice following anterior chamber inoculation. Inoculation of the abdominal subcutaneous tissue rarely results in progressive tumor growth.

Two experiments were devised to determine whether this difference in tumor growth is due to an adaptation of the tumor cells to the host during

the period of anterior chamber growth, or to a difference in host resistance between the cranial and the abdominal subcutaneous tissue.

Tumors which had extensively invaded the subcutaneous tissue of the head and neck following anterior chamber inoculation were re-transplanted to the abdominal subcutaneous tissue of C57 brown mice. The incidence of tumor growth was compared to that of another group of mice in which the tumor had been re-transplanted from subcutaneously inoculated donors. Had adaptation of tumor cells to the host taken place, growth in the former group should have exceeded that in the control group. There was no evidence of tumor cell adaptation.

To determine whether the host resistance of the cranial subcutaneous tissue differed from that of the abdominal subcutaneous tissue, the tumor was inoculated conjunctivally in one group of mice, and to the facial subcutaneous tissue in another.

While tumor growth occurred more commonly in these two groups than in the subcutaneous (abdominal) controls, the incidence was still significantly below that in the anterior chamber controls.

STUDIES OF STABLE AND RADIOACTIVE LANTHANUM IN NORMAL AND TUMOR-BEARING MICE. Daniel M. Ekstein* and Ruth Lewin,* with the technical assistance of Leonard Woidowsky. (Radioisotope Laboratory, Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

Lanthanum ions, as well as those of other rare earths, form insoluble complexes with nucleic acids. Because of the increased radiation sensitivity of rapidly dividing cells and the wide variety and radiation characteristics of the available rare earth isotopes, their study in animals bearing tumors was indicated. Preliminary to therapeutic experiments, distribution studies in normal and tumor-bearing mice were performed, with radioactive lanthanum-140 as a tracer in stable lanthanum. The lanthanum was administered by subcutaneous and intravenous routes. The animals were sacrificed after suitable time intervals and various tissues analyzed for lanthanum. It was found that subcutaneously administered lanthanum remained unabsorbed. In the animals injected intravenously, approximately 50 per cent of the lanthanum was absorbed from the blood stream within 1 hour. After 1 day, more than 75 per cent was found in the liver and spleen. Thereafter, the amount in liver and spleen decreased, whereas lanthanum uptake in bone increased. The concentration of lanthanum in spontaneous mammary adenocarcinoma was similar to the average carcass concentration. The MLD_{50} of stable lanthanum has been estimated to be less than 0.08 mg. lanthanum ion per gram mouse. At toxic levels, cytological and cytochemical evidence of severe liver cell damage has been demonstrated. Further studies on the effect of radiolanthanum upon tumor-bearing animals will be reported.

EFFECT OF SOME SUBSTITUTED 2,6-DIAMINOPURINES ON THE GROWTH OF SARCOMA 180. G. B. Elion,* S. M. Buckley,* C. C. Stock, and G. H. Hitchings.* (Department of Biochemistry, The Wellcome Research Laboratories, Tuckahoe, and Division of Experimental Chemotherapy, The Sloan-Kettering Institute, New York, N.Y.)

Of nineteen substituted 2,6-diaminopurines tested against Sarcoma 180 in mice, five compounds have shown some inhibitory activity against the tumor. The inhibitory effects of these purines on the growth of *Lactobacillus casei* and the reversal of these inhibitions by folic acid and by adenine also have been studied. These compounds may be graded according to the ease with which the inhibitory effects on the lactobacillus are prevented by added folic acid. In general, those purines with which this reversal is more difficult have stronger effects against the sarcoma.

INFLUENCE OF 2-ACETYLAMINOFLUORENE ON THE METABOLISM OF FAT AND PROTEIN BY THE RAT. R. W. Engel. (Department of Animal Husbandry and Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

The inclusion of 2-acetylaminofluorene in low fat diets greatly inhibited the growth rate of weanling rats. Such diets produced tumors in the orbital cavity in approximately 30 per cent of the animals that survived for 32 weeks or longer. This type of tumor was produced only very rarely on animals receiving similar diets containing 20 per cent of lard.

The marked growth retardation observed with the low fat diets was accompanied by a marked reduction in the efficiency of protein utilization for growth and by an apparent increased dietary requirement for essential fatty acids. With a low fat diet containing 12 per cent of protein, the inclusion of 2-acetylaminofluorene at a level of 0.03 per cent reduced the protein efficiency (grams body weight gain per gram of protein consumed)

from 1.1 to 0.75. With a diet containing 21 per cent of protein, the same level of carcinogen reduced the protein efficiency from 0.50 to 0.18. The protein efficiency was not influenced by 2-acetylaminofluorene when the diets contained 20 per cent lard.

Rats receiving the low fat diets plus 2-acetylaminofluorene developed essential fatty acid deficiency lesions more readily than did rats receiving similar diets without the carcinogen.

These results indicate that the ingestion of 2-acetylaminofluorene is concerned with the metabolism of proteins and fats by the rat and that an absence of dietary fat promotes the induction of tumors of the orbital cavity.

CARCINOGENIC HYDROCARBONS IN PROCESSED RUBBER AND IN CARBON BLACK. Hans L. Falk,* Paul E. Steiner, and Sam Goldfein.* (Department of Pathology, University of Chicago, Chicago, Ill.)

Benzene extracts of processed rubber (an automobile tire and rubber stoppers), investigated because of their strong fluorescence, were found to be carcinogenic on the skin of mice. By chromatography and ultraviolet spectrophotometry 3,4-benzpyrene, a strong carcinogen, and seven additional aromatic hydrocarbons of weak or unknown carcinogenicity were detected (*Cancer Research*, in press). Of the raw materials used in the processing of rubber, carbon black is the most likely source of these hydrocarbons and it was therefore further investigated. The fluorescent extracts of some carbon blacks yielded all the aromatic hydrocarbons found in rubber and, in addition, coronene, which had not been found in the rubber extracts.

Nearly all the compounds detected contain four pyro-condensed benzene nuclei. In ascending order of complexity they are: pyrene, 3,4-benzpyrene, 1,2-benzpyrene, anthanthrene, 1,12-benzperylene, and coronene. In addition, the simpler compounds cyclopentenophenanthrene, chrysene, and a benzanthracene derivative were found. 3,4-Benzpyrene is known to be a strong carcinogen, and pyrene, chrysene, and 1,2-benzypyrene as weakly carcinogenic. The remaining compounds are inactive or have not been tested for carcinogenicity.

Only three types of carbon black have been investigated; all are furnace blacks. Two of them, of large particle size, possessed strong fluorescence and contained all the hydrocarbons described above. The third, of small particle size, showed no fluorescence and contained only pyrene and the benzanthracene derivative. A knowledge of the factors responsible for the presence of carcinogens in carbon black will help evaluate the possible health hazards in the industries concerned.

THE INTERRELATIONSHIPS OF NITROGEN, PHOSPHORUS, AND POTASSIUM IN SOME NEOPLASTIC DISEASES. Leonard D. Fenninger* and Christine Waterhouse* (introduced by G. Burroughs Mider). (University of Rochester School of Medicine and Dentistry, Rochester, N.Y.)

Exchanges of nitrogen in the metabolism of protoplasm must be ultimately associated with exchanges of phosphorus and potassium in amounts comparable to those which exist in tissue. Metabolic studies of patients with malignant neoplasms and related diseases which are growing progressively or regressing because of effective therapy indicate relationships between nitrogen, phosphorus, and potassium exchanges which differ from those observed in normal subjects. This suggests that the chemical composition of tumor protoplasm is different from that of normal tissue.

Neoplastic tissue from two patients with acute leukemia and from one each with chronic myelogenous leukemia, lymphosarcoma, and Hodgkin's disease contained 1.9–2.6 times as much phosphorus and 1.5–1.9 times as much potassium as does normal tissue.

An equation based on these studies was derived to determine the change in the partition of nitrogen between the host and its neoplasm. Application of this formula to the observed phosphorus and nitrogen balances in a patient with acute leukemia indicated that all the ingested nitrogen retained and additional nitrogen derived from the host tissue were used for tumor formation. The rate of tumor growth thus calculated correlated well with observed clinical and autopsy findings. Retention of potassium was excessive in relation to nitrogen retention and greater than that accounted for by tumor growth.

Similar studies in a patient with lymphosarcoma indicated that excess nitrogen excreted prior to therapy was supplied from host tissues. The nitrogen lost during the induction of a clinical remission by ACTH was derived almost exclusively from neoplastic cells.

EFFECT OF 8-AZAGUANINE ON GROWTH AND RESPIRATION OF TUMOR TISSUE. Murray Finkelstein,* Wallace D. Winters,* Paul A. Thomas,* Clarke Davison* and Paul K. Smith. (Department of

Pharmacology, The George Washington University School of Medicine, Washington, D.C.)

The guanine analogue, 8-azaguanine, is known to inhibit the growth of certain tumors but to have little or no effect on others. The present report is a study of the effect of this compound on the growth and respiration of Sarcoma 37 in CAF_1 strain mice.

Subcutaneous injections of five to seven doses of 100–175 mg. of 8-azaguanine per kilogram, begun on the day of or within 4 days of tumor implant, were effective in delaying tumor growth. This produced anorexia and marked wasting. Gross post mortem examination showed only a diminution in size and discoloration of the spleen. Histologically, the spleen showed cellular degeneration. Whereas sections of tumors of these mice showed few changes, sections of tumors from mice that received a single dose of 500 mg/kg showed marked hemorrhagic areas and cellular degeneration with pyknotic nuclei, along with a marked decrease in mitosis after 24 hours.

In concentrations up to 0.1 per cent suspension, 8-azaguanine had no effect, *in vitro*, on the respiration of tumor, kidney, liver, brain, or spleen slices. Tumor slices removed at various intervals after a subcutaneous dose of 500 mg/kg showed a gradual diminution in the respiration to almost zero after 24 hours, with partial to complete recovery after 48 hours. This was an apparently selective action, for the respiration of brain, liver, kidney, and spleen was not altered. The rates of respiration of tumor and spleen of mice that received the daily doses of 8-azaguanine were not significantly different from those of untreated control mice. Doses smaller than 200 mg/kg produced no inhibition of respiration.

Guanine, in doses up to 1,500 mg/kg, was without effect on the rate of respiration of tumor slices and did not significantly alter the inhibitory effect of 500 mg. of 8-azaguanine/kg.

β-GLUCURONIDASE ALTERATIONS IN HUMAN CERVICAL AND BREAST CANCER. William H. Fishman, Freddy Homburger, S. Charles Kasdon,* and Charles D. Bonner.* (Cancer Research and Cancer Control Unit, Departments of Surgery, Medicine and Biochemistry, Tufts College Medical School, Boston, Mass.)

Tissue biopsies of carcinoma of the cervix are uniformly rich in β-glucuronidase activity, as compared to biopsy specimens taken from benign lesions of the uterine cervix and from healthy cervices (*J.A.M.A.*, **144**:892, 1950). The vaginal fluid, however, showed a significant elevation in β-glucuronidase titers only in premenopausal women with untreated carcinoma of the cervix. Since the vaginal fluid enzyme activity appeared to be associated with ovarian function, as evidenced by the greater incidence of high β-glucuronidase values in post-menopausal and in castrate women, a study has been made of the vaginal fluid β-glucuronidase in relation to the menstrual cycle. In some 40 complete cycles studied in 20 women, a low range of activity was found at midcycle. A complete study of vaginal fluid β-glucuronidase in a subject before and after panhysterectomy and bilateral oöphorectomy, followed by successive courses of estrogen and progesterone, is presented. In other studies, the effect of oral stilbestrol on the vaginal fluid β-glucuronidase was investigated. As a rule, the activity of the enzyme in vaginal fluid was depressed following stilbestrol administration.

Serum β-glucuronidase was observed to increase during and after the administration of stilbestrol to post-menopausal women (5 mg/day for 21 days). Similar results were seen in several women with cancer of the breast receiving methylandrostenediol or stilbestrol or both for prolonged periods of time. The physiological and clinical significance of these data is discussed.

THE RELATIONSHIP OF NITROFURAZONE TO THE ADRENAL CORTEX AND ITS EFFECT ON TUMOR GROWTH. Charles E. Friedgood* and Charles B. Ripstein* (introduced by E. V. Cowdry). (Department of Surgery, Maimonides Hospital, and The State University Medical Center at New York, N.Y.)

Nitrofurazone has been previously reported to cause adrenal cortical hypertrophy and to retard the growth of a fibrosarcoma in mice. Morphologically, the hypertrophy of the adrenal cortex was further investigated with special stains. Sudan IV gave a more intense color reaction, owing to the increased lipid content. Silver nitrate stain for ascorbic acid also yielded an increased reaction, and Ashbel-Seligman's carbonyl stain showed a higher concentration of the stain in the fascicular zone of the adrenal cortex. Slides will be projected of these stains. The drop in total circulating eosinophile count observed in the treated animals was comparable to the response obtained with epinephrine and cortisone.

Nitrofurazone increased the life-span of C3H mice with fibrosarcoma S-13 from an average of 21 days to 38 days. Since adrenal cortical hypertro-

phy was observed in the nitrofurazone-treated tumor-bearing mice, we treated a group of adrenalectomized tumor-bearing mice with the compound. Nitrofurazone given to these mice was ineffective in retarding the growth of the tumor. This group lived an average of only 19 days.

The mode of action of nitrofurazone may be through the adrenal cortex.

ORGAN BLOOD VOLUMES OF TUMOR-BEARING MICE: OLIGEMIA OF NEOPLASMS. J. Furth, E. J. Beale,* L. Wish,* and M. M. Knoohuizen.* (Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.)

The cell and plasma volumes of organs and tumors can be accurately determined by isotopic technics. Such determinations made in mice bearing granulosa tumors with hypervolemia led to the discovery that tumors are relatively bloodless. The following are illustrative average values: (*a*) In 24 normal mice the large vessel hematocrit averaged 42.9 per cent. The liver contained 28.5 per cent blood (organ hematocrit, 43 per cent); the remaining 71.5 per cent will be called parenchyma. The spleen had 35.7 per cent blood (organ hematocrit, 70.6 per cent) and 64.3 per cent parenchyma; the kidney, 24.4 per cent blood (organ hematocrit, 39.6 per cent) and 75.6 per cent parenchyma. (*b*) Sixteen grafted granulosa tumors contained 95.6 per cent parenchyma and only 4.4 per cent blood (tumor hematocrit, 21.0 per cent). (*c*) Six grafted breast tumors averaged 6 per cent blood (tumor hematocrit 20 per cent) and parenchyma 94 per cent.

It is possible that tumors have a sluggish circulation. However, extending the mixing time of the injected labeled material from the conventional 6 to 30 minutes still yielded low tumor blood values. The normal spleen contains more erythrocytes than plasma (hematocrit, 70.6 per cent). In hypervolemia this drops to 25.6 per cent as the spleen takes up some of the excess plasma. More plasma is stored in the liver, in which blood averages 22.5 per cent of the total blood mass of the animal (normal, 18.9 per cent). These determinations point to other hitherto unrecognized blood reservoirs in hypervolemia. The oligemia of tumors is of special interest in relation to the well known anomalous metabolism of neoplasms.

THE EFFECTS OF FREEZING, THAWING, AND LYOPHILIZATION UPON THE TRANSPLANTABILITY OF MOUSE LEUKEMIC CELLS. Ronald M. Gabrielson* and Jerome T. Syverton. (Department of Basteriology and Immunology, University of Minnesota, Minneapolis, Minn.)

Leukemic cells from F strain mice were employed as two lines of lymphoid leukemia, 876 and 926, and one line of myeloid leukemia, 765. Cellular suspensions of splenic tissues, mixed in equal parts with 5.3 per cent glucose to yield a 50 per cent suspension, were treated (*a*) by freezing and thawing rapidly, (*b*) by freezing and thawing slowly, and (*c*) by lyophilization. Methyl cellosolve-dry ice and ether dry ice were utilized for freezing mixtures. Rapid treatment consisted of sudden immersion to −76° C., at which temperature it was maintained for intervals of 12, 24, 36, 48, 60, or 72 hours when the test suspension was thawed rapidly by immersion at 37° C. Slow freezing was accomplished at 4° C., with progressive lowering of the temperature by addition of crushed dry ice to −76° C. The process of slowly thawing each mixture consisted in the transfer successively to temperatures of 4° C., 20° C., and 37° C. The results made it apparent on tests for viability by transplantation of the treated leukemic cells to F_1 hybrid mice (*a*) that freezing and thawing slowly had little or no effect upon the viability of the cells; (*b*) that, in contrast to these findings, "fast freeze-fast thaw" resulted in total loss of viability when tested during the first 24 hours after treatment, partial to total loss in the period from 36 to 60 hours, and positive transfer at 72 hours in 31 of 54 recipients. Moreover, the fast-frozen material retained its ability to transmit leukemia after storage at −18° C. for 526 days. Studies employing liquid N_2 and O_2 for freezing are under way. Lyophilization from the frozen state resulted in total inactivation as measured by transmissibility.

THE EFFECT OF SUBSTITUTED MALONONITRILES ON THE GROWTH OF TRANSPLANT TUMORS IN MICE. Emery M. Gal, Harriett Ezra,* F. H. Fung,* and David M. Greenberg. (Division of Biochemistry, School of Medicine, University of California, Berkeley, Calif.)

Preliminary studies on the effects of substituted malononitriles of the type R-CH:C$(CN)_2$ and of the parent compound were previously reported by us (*Cancer Research*, **10**:221, 1950). At present, over 40 malononitriles of the above type have been tested in strain A, C3H, and C57 mice bearing the tumors S-37, C3H-S, 6C3HED, C57-E 0771, and myeloid leukemia (C1458), respectively. The compounds were synthesized by the authors, and the choice of substitutions was determined by steric relations as well as by resemblance to groups pres-

ent in colchicine and podophyllin. None of the compounds tested produced regression or complete arrest of growth. Many of the compounds, however, significantly slowed down the rate of growth, among them notably the 5-nitrofurfural, *p*-nitrobenzal and piperonal malononitriles. It was found that these malononitriles caused about 50–70 per cent retardation of the growth (in younger animals even higher) of sarcoma S-37 and carcinomas C3HS and C57-E 0771. They had no effect on leukemia, nor did they significantly influence survival time. The compounds were dissolved in sesame oil and were administered intraperitoneally. Attention has been given to the influence of food intake and to the weight losses of the animals. Statistical analysis of the data showed that the effects observed cannot be correlated with the weight loss of the animals. It was found that neither sodium cyanide, malononitrile, malonic acid, thiocyanate, nor the parent aldehydes produced any effect on the rate of tumor growth, with the exception of the 5-nitrofurfural which caused an approximate 25 per cent arrest of growth. In order to determine the effect of replacement of the $:C(CN)_2$ group, we have investigated the action of *p*-nitrobenzal and 5-nitrofurfural thiosemicarbazones. These had no effect on tumor growth. Studies on the mechanism of action both *in vivo* and *in vitro* are in progress.

PARATHYROID HYPERPLASIA IN EARLY INFANCY. Lytt I. Gardner (introduced by Charles W. Hooker). (Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Md., and Department of Pediatrics, Harvard Medical School, Boston, Mass.)

"Idiopathic" parathyroid hyperplasia in young infants has been described by Kaplan (*Arch. Path.*, **34**:1042, 1942). In view of dietary Ca and P content as a possible etiologic factor, dietary histories have been investigated in a number of Kaplan's cases, and the microscopic sections have been kindly made available by Dr. Sidney Farber for review. Only cases with a reliable dietary history and no renal damage were included.

Normal parathyroids were noted in eight infants, all less than 29 days of age. Of this group, four had been fed nothing but human milk, one received human milk with a cow's milk supplement, and three received no oral feedings.

Hyperplastic parathyroid glands were found in eight infants, all less than 32 days of age. Of these, seven had been fed cow's milk formulas, and one received no oral feedings.

Four older children, from 4 months to $10\frac{1}{2}$ years, all showed normal parathyroid glands. Of these, one had been breast-fed, two had received cow's milk formulas, and one had received both human and cow's milk.

The above findings are of interest in view of: (*a*) the relatively high inorganic phosphate content of cow's milk, (*b*) the relative inability of the very young infant's kidney to clear phosphate, and (*c*) the experimental evidence that parathyroid hyperplasia can be produced by injection or ingestion of excess phosphate. Although the data show inconsistencies, there is a suggestive relationship between cow's milk feeding in early infancy and parathyroid hyperplasia. In later infancy and childhood, maturation of renal function with respect to phosphate clearance would make such hyperplasia less likely.

CELL STRUCTURE: A COMPARISON OF PHASE AND ELECTRON MICROSCOPY. G. O. Gey and F. B. Bang.* (Departments of Surgery and Medicine, The Johns Hopkins Medical School and Hospital, Baltimore, Md.)

Cultured normal and malignant cells of the same histogenic origin provide excellent material for comparative cytological studies of the living and fixed cells. In thinly spread areas, portions of cells show, under phase microscopy and with the aid of motion pictures, a definitive endocellular traffic, apparently due to the activity of the streaming hyaloplasm. High power observations of such living cell components as plasma cell membrane, filamentous and membranous processes, mitochondria, and inclusion vacuoles allow a good interpretation of electron microscope images of comparable structures studied in the fixed cell at still higher powers. Emphasis will be placed on the ability of cells to produce membranous and filamentous processes and to ingest and discharge particles. The relation of the delicate filamentous processes and other fibrous components to the hyaloplasm and the character of the intracellular traffic will be described. The filamentous plastic mitochondria can be demonstrated to break, to fuse, to be passively swept down separate channels, and lateral buds may be pulled off by contact with contracting elements.

Some of the differences between specific rat malignant cells (T-333) produced in continuous cultures and their normal prototypes (14p), as determined by this study, will be pointed out and compared with other continuously cultured cell types. The smaller and thinner normal prototype cell (14p) produces more stable processes. The more rapidly growing malignant cells (T-333) accumulate a massive ring of fat droplets, and many de-

velop a large juxta-nuclear mass, so rigid as to bend the large nucleus. Abnormal cleavages occur with much greater frequency in the malignant cells.

ELEMENTAL SULFUR AND TUMOR EXTRACT AS INHIBITOR OF PASTEUR EFFECT IN YEAST. D. Ghosh* and H. A. Lardy* (introduced by H. P. Rusch). (Department of Biochemistry and Institute for Enzyme Research, University of Wisconsin, Madison, Wis.)

In the course of investigation relating to isolation and identification of a metabolic regulator in mammalian spermatozoa, various other animal tissues, including malignant tumors, were analyzed for similar regulatory principles. Acetone powders of fresh or frozen tissues were subjected to mild alkaline hydrolysis and subsequently extracted with chemically pure carbon tetrachloride at acid pH. The extracts were tested for activity by their ability to inhibit Pasteur effect in baker's yeast. Extracts from normal tissues except the testis were inactive, while those from all the different types of human and rat tumors tested showed a very high degree of activity. Liver extract from fetal calf was slightly active; kidney extract was inactive.

The yeast-active principle was isolated in crystalline form from hog testis and was identified as elemental sulfur. Catalytic amounts of sulfur (0.4 μg/ml of yeast fermentation media) increased aerobic fermentation to the anaerobic level without affecting respiration. Elemental sulfur was readily reduced by yeast to H_2S, which produces a similar inhibition of the Pasteur effect in yeast. Glutathione and cystine were inactive; cysteine showed activity only after a preincubation period sufficient to liberate H_2S and free S by the cysteine desulfurase of yeast. Under the conditions of hydrolysis employed, neither cystine nor cysteine was decomposed to give any activity in CCl_4 extract; glutathione was, however, partially decomposed. In the absence of any significant difference in the total sulfur content between normal and malignant tissue the behavior of the latter, as well as of spermatozoa and testis, is believed to reflect a difference in structure of sulfur-containing proteins in these tissues.

FURTHER STUDIES ON THE RELATIVE BIOLOGICAL CHARACTERISTICS OF ANALOGOUS MAMMARY TUMORS. Anna Goldfeder. (Cancer Research Laboratory, Department of Hospitals, City of New York, and the Department of Biology, Graduate School of Arts and Science, New York University, N.Y.)

Previous investigations have shown that mammary tumors, diagnosed as adenocarcinomas and histologically almost identical, differed significantly in their rate of growth, metabolic activity, and radiosensitivity. It was endeavored to identify and determine quantitatively the factors responsible for the difference in the genetic make-up of these tumors.

Studies were carried out along three lines: (*a*) mitotic activity, (*b*) intermediary metabolism, and (*c*) radiosensitivity.

a) It was found that mitotic activity is not the only factor determining tumor size, as measured externally. The secretory activity, as evidenced by engorgement of the glandular structures and the presence of mucoid material, proved to be an additional factor influencing tumor size.

b) The levels of phosphorylated intermediates were taken as a measure of the intermediary metabolism. A significant difference was noted in the amounts of adenine compounds in two analogous mammary adenocarcinomas. The faster growing tumor proved to have a larger quantity of adenosine triphosphate, indicating that a higher energy level is available in this tumor.

c) The degree of differentiation proved to be a factor in determining radiosensitivity. The inherent potentiality of a tissue to differentiate can be enhanced by radiation, as evidenced by keratinization of epithelial cells and pearl formations.

It will be shown that the above-mentioned characteristics may differ significantly, not only among mammary adenocarcinomas arising in different strains of mice, but even among those arising in the same inbred strain.

THE CITROVORUM FACTOR AND THE REVERSAL OF AMINOPTERIN INHIBITION IN *DROSOPHILA MELANOGASTER*. E. D. Goldsmith and Morris H. Harnly. (Department of Histology, College of Dentistry, and Washington Square College of Arts and Sciences, New York University, New York, N.Y.)

Larvae of the fruit fly maintained on a yeasted Pearl's synthetic medium containing aminopterin (4-aminopteroylglutamic acid, a PGA antagonist), showed a retarded rate of development and lowered survival values. The per cent emergence (to 0 per cent) varied inversely with the concentration of aminopterin. It has been reported previously (Goldsmith and Harnly) that DNA (thymus nu-

cleic acid, Schwarz) and PGA (Lederle) have proved effective, and thymine only partially so, in overcoming the antagonist effect on the fruit fly.

Incorporation of 5–50 mg. per cent of the citrovorum factor in the Pearl's medium containing 2.5 and 5.0 mg. per cent of aminopterin has resulted in various stages of reversal of the antagonist. Preliminary observations indicate that suitable concentrations of the citrovorum factor can reverse the aminopterin action completely and that it appears to be more effective than PGA in this respect. The work is being continued; other factors and combinations are under investigation.

THREE PHENOTYPICALLY SIMILAR PIGMENT-CELL TUMORS WITH THREE DIFFERENT GENOTYPES. Myron Gordon, Ross F. Nigrelli, and Sophie Jakowska. (New York Aquarium, New York Zoölogical Society, and College of Mount Saint Vincent, New York, N.Y.)

The hereditary history of black and red tumors in a series of platyfish-swordtail hybrids was traced to the linkage of two genes: *Sp* for macromelanophores and *Rt* for xanthoerythrophores. Somewhat similar tumors were found rarely in other hybrids with the following gene combinations: *Sr* and *Dr; Sp* and *R*. *Sr* is a sex-linked allele of *Sp*, and, like *Sp*, it controls the development of macromelanophores. *Dr* and *R* are sex-linked alleles of *Rt* and, like *Rt*, control the development of xanthoerythrophores. The genes of both series differ from each other with reference to the color patterns which they produce in the platyfish, and with reference to the frequency with which they, in combination, produce erythromelanomas in the platyfish-swordtail hybrid.

The red growths in *SpRt* and *SpR* hybrids, which occurred in the head region, showed striking similarities. In both, xanthoerythrophores were involved. Such tumors were identified as xanthoerythrophoromas. In certain stages of development of these tumors, melanotic cells from other regions of the body may invade the growth, producing a condition referred to previously as erythromelanoma, or, strictly speaking, a xanthoerythromelanoma. Eventually, as in the case of tumors in *SpRt* hybrids, the xanthoerythrophoroma may be invaded, destroyed, and replaced by the more malignant melanoma. In the *SrDr* hybrid, the red tumor occurred in the tail region. It was sarcomatous in appearance, consisting mainly of spindle-shaped cells and a relatively few xanthoerythrophores. In this tumor, typical macromelanophores were also present. All the growths showed invasive tendencies.

MORPHOLOGICAL AND CYTOLOGICAL EFFECTS OF CARCINOGEN-PROTEIN CONJUGATES IN THE DEVELOPING FROG'S EGG. Elizabeth Ufford Green and E. Marie Hearne Creech.* (Institute for Cancer Research and Lankenau Hospital Research Institute, Fox Chase, Philadelphia, Pa.)

Hydrocarbon-protein conjugates, provided by Dr. Hugh J. Creech, when applied in solution to fertilized eggs of *Rana pipiens* and *Rana palustris* have been found capable of arresting the development of the eggs and embryos. The most active compound used—9,10-dimethyl-1,2-benzanthranyl-3-carbamido horse serum albumin—will cause regression of the first cleavage furrow and failure of further development in a concentration of 37 μg. of hydrocarbon per milliliter of solution (1.4×10^{-4} M hydrocarbon) while 1 μg. per milliliter (4×10^{-6} M hydrocarbon) will arrest development before or soon after the beginning of gastrulation. At the latter concentration, cleavage of the surface cells of the animal hemisphere is arrested, while cells in the interior continue to divide for some time, and internal structures are formed whose nature will be discussed. Cytological studies of these arrested gastrulae showed large or multiple nuclei in the arrested surface cells, while the chromosomes of dividing cells showed degeneration, clumping, fragmentation, polyploidy, precocious splitting, and separation of sister chromatids without spindle formation (the paired chromatids sometimes lying parallel and sometimes forming rings and crosses). Lower concentrations of hydrocarbon permitted development to continue. However, tail tips from tadpoles reared in a solution sufficiently dilute to produce no gross abnormalities still showed abnormalities of chromosome behavior (particularly precocious separation of the chromatids, though polyploidy, fragmentation and lagging were also found), and this was true in regenerated tail tips in tadpoles which were kept for 8 days in spring water.

THE RELATIONSHIP OF FOLIC ACID (FA) AND CITROVORUM FACTOR (CF) TO THE TOXICITY OF AMINOPTERIN. Ezra M. Greenspan,* Abraham Goldin, and Emanuel B. Schoenbach.* (Clinical Research Unit of the National Cancer Institute, U.S. Marine Hospital, and the Department of Preventive Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md.)

A defect in the ability to utilize FA was demonstrated when mice received repeated doses of FA

and aminopterin. Animals receiving a single lethal dose of aminopterin survived when either FA or CF was administered. CF could protect mice when aminopterin was repeatedly administered. Mice protected by CF could not tolerate an additional dose of aminopterin together with FA. Aminopterin appeared to produce, among mice protected by CF, a marked deficiency in a mechanism concerned with the conversion and utilization of FA. The relationship of dose-mortality responses observed following the administration of either FA or CF with aminopterin will be discussed with respect to the postulated enzyme systems involved.

INDUCTION OF TUMORS WITH NITROGEN MUSTARDS. A. Clark Griffin, Eugenia L. Brandt,* and E. L. Tatum.* (Departments of Chemistry and Biology, Stanford University, Calif.)

The somatic mutation theory of cancer is supported by the demonstrated mutagenic activity of certain carcinogenic agents such as methylcholanthrene, dibenzanthracene, benzypyrene, and also ultraviolet radiation. This theory would also demand that the nitrogen mustards, which have been shown to be mutagenic and to possess characteristic radiomimetic and nucleotoxic activities, would likewise be carcinogenic. This study was therefore initiated to test further this hypothesis by examining the two nitrogen mustards, methyl-bis(β-chloroethyl)-amine (HN2) and tris (β-chloroethyl) amine (HN3).

Swiss mice and albino rats were injected intravenously, subcutaneously, or intraperitoneally for 1–40 weeks with either HN2 or HN3. Animals were sacrificed and examined at autopsy when they appeared moribund or when gross tumors were evident. All remaining animals were also sacrificed at the end of approximately 1 year. A high incidence of tumors was found in nitrogen mustard-treated mice. The first observable tumors appeared approximately 6–7 months after the experiments were initiated. Subcutaneous administration was found to be most effective when only one dose was given; however, in multiple weekly injections, all three routes produced similar tumor incidences. Injection of only one dose was found to be as effective as continued treatment. Tumors produced were of three main types: adenocarcinoma of lungs, leukemia or lymphosarcoma, and sarcomas. Angiomas, angiosarcomas, and osteogenic sarcomas were also noted. The demonstration that the nitrogen mustards will induce tumors appears to be consistent with the somatic mutation theory of cancer.

THE DISTRIBUTION OF PARENTAL RADIOACTIVE GOLD COLLOID (Au^{198}) IN THE RAT. Mary Lynn Haigler* and George Z. Williams. (Samis Grotto Cancer Research Laboratories, Department of Oncology, Medical College of Virginia, Richmond, Va.)

Little experimental data is available concerning the details of distribution and disposition of radioactive gold colloid in animals.

Radiogold sol (Au^{198}) obtained from Dr. Tabern at the Abbott Research Laboratories was injected in doses from 10.0 μc. to 3 mc. in albino rats by intracardiac, intraperitoneal, intrasplenic, intramuscular, and subcutaneous routes. The animals were maintained on a normal complete diet in metabolism cages, and all urine and stools were separately collected each 24-hour period and counted for radioactivity. Blood was collected and counted periodically. Results are expressed as percentage of total activity administered.

The effects of various agents on the uptake and distribution of the gold were determined.

Biological half-life was unlimited. Less than 2 per cent of the total injected amount by any route was excreted during the period of measurable activity of the substance. The amount taken up by the *liver* when gold is administered by various routes was as follows: intracardiac, 25 per cent; intrasplenic, 21 per cent; intraperitoneal, 4.1 per cent; intramuscular, 2.3 per cent; subcutaneous, 1 per cent or less.

Hyaluronidase increases the amount of radiogold circulating in the blood stream after intramuscular and subcutaneous injection to 10 per cent and 25 per cent, respectively. Heparin, india ink, and silica were found to have no effect.

Dialysis of the radioactive sol through semipermeable cellophane membranes yields 0.16–0.37 per cent gold in the dialysate, which is of the same order as the amount excreted by the kidney.

Distribution in the liver as determined by radioautographs is uniform. The sol is phagocytized by the reticulo-endothelial cells of the liver, spleen, lung, kidney, and bone marrow; 90–95 per cent is taken out of the blood by the liver and the spleen, very small quantities going to reticular elements elsewhere.

DIURNAL RHYTHM IN TAIL BLOOD EOSINOPHIL LEVELS. Franz Halberg,* John J. Bittner, and Maurice B. Visscher. (Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

The absolute numbers of circulating eosinophils were determined under standardized conditions at two different periods of the day in 4–7-month-old female mice. The animal material consisted of several inbred stocks with different incidences of spontaneous mammary cancer. Groups of mice from the same stock with and without the mammary tumor milk agent were studied.

The diurnal rhythm in circulating eosinophils, as observed in the absence of stress other than the animals' activity under undisturbed conditions, appears as a physiological phenomenon in mature inbred mice of both sexes.

When pregnant and nonpregnant animals of several stocks were compared, the results indicated the presence of a diurnal rhythm in those of each group. A significant eosinopenia of the third trimester of pregnancy was also noted.

CHANGES IN THE RADIOSENSITIVITY OF TUMOR FRAGMENTS INDUCED BY PRE-TREATMENT *IN VITRO* WITH CYSTEINE, METHYLENE BLUE, AND SODIUM CYANIDE. B. VINCENT HALL* (introduced by Austin M. Brues). (Biological and Medical Division, Argonne National Laboratory, Chicago, and the Department of Zoology, University of Illinois, Urbana, Ill.)

Fresh tumor fragments of a vigorously growing, anaplastic mouse carcinoma were pre-treated for 20 minutes with cysteine, methylene blue, or NaCN solutions, and then irradiated in culture dishes under standard conditions (200 kv, HVL 8 mm. Al, dose rate 130 r/min, t.d., 12.7 cm). The tumor fragments were pooled and mixed before segregation into groups. Comparison of the number of implants which fail to grow with the growth rates of the surviving implants indicates the effect of pre-treatment upon radiosensitivity. Solutions of 1.25 mg. cysteine per cubic centimeter enabled 58 per cent of the implants to survive 4,000 r, whereas none of the control tumor fragments, irradiated in 0.85 per cent NaCl, survived. The growth of non-irradiated tumors was not influenced by cysteine at this concentration. Pre-treatment of tumor fragments with 0.01 or 0.001 M NaCN or methylene blue, on the other hand, greatly decreased the number of surviving implants. Treatment of non-irradiated tumors with NaCN, or methylene blue solutions of this concentration, had little effect upon the number of subsequent takes, but the more concentrated solutions tended to retard the initial growth of the implants. The decreased tumor radiosensitivity afforded by pre-treatment *in vitro* with cysteine paralleled effects observed *in vivo*. The other agents, which under certain circumstances were protective *in vivo*, rendered the tumor more sensitive, suggesting either that they act through different mechanisms or that a summation of injuries is involved.

NUTRITIONAL AND TEMPERATURE EFFECTS ON THE FREQUENCY OF AN HEREDITARY MELANOMA AND *DROSOPHILA MELANOGASTER*. MORRIS H. HARNLY, F. FRIEDMAN,* G. C. EMERY,* and E. GLASSMAN.* (Washington Square College, New York University, New York, N.Y.)

Genetic tests have shown that this hereditary melanoma is due to a recessive gene in the second chromosome. When multiples of 5–400 newly hatched larvae are introduced simultaneously into each vial 85 of 100 adults have one or more melanomas. With two phenotypes, daily insertions for 8 days demonstrate that 85 per cent of the larvae introduced on the second and third day develop tumors; 49 per cent of those introduced on the fifth to eighth day develop tumors; 47 per cent are tumorous when introduced on the seventh to eighth day; and of those placed in the vials on the eighth day of 100 larvae/vial/day for 8 days, only 33 per cent develop tumors. The progressive drop in survival and the increasing delay in development with later entrance to the vials, together with these changes in tumor incidence, demonstrate that poor nutrition inhibits tumor formation and suggest that nutritional conditions during the first instar are critical to subsequent tumor formation. Preliminary experiments suggest that vitamin components may be one of the nutritional factors. These points are now under investigation. With optimum food conditions, tumor frequency increases from 24 per cent at 14° C. to 85 per cent at 21°, declines steadily to 21 per cent at 30°, and rises again to 96 per cent at 32°. Tumor size and multiplicity differ at the optimum temperatures of 21° and 32° C. Data suggest that the rate of development may be a factor in these shifts in tumor frequency with temperature. This point is under investigation.

THE FATTY ACID COMPOSITION OF RATS GROWING WALKER CARCINOMA 256. FRANCES L. HAVEN, W. R. BLOOR, and CHALLISS RANDALL.* (University of Rochester School of Medicine and Dentistry, Rochester, N.Y.)

The fatty acids of Walker carcinoma 256 and of rats bearing this tumor were compared with the fatty acids of pair-fed rats without tumors on diets containing Crisco and elaidin. The percentage of

solid and liquid fatty acids and the degree of unsaturation of each fraction were determined.

Tumors from rats in both dietary groups were significantly higher in liquid fatty acids than the carcasses of the host rats. An effect of tumor on the host was evident, since the liquid fatty acids of the carcass of rats with tumors were more unsaturated than the liquid fatty acids of pair-fed rats without tumors.

The ratio of approximately 45 to 55 for solid to liquid fatty acids of carcasses of tumor-bearing rats and of pair-fed controls on Crisco diet was significantly altered in rats receiving elaidin. The carcasses of tumor-bearing rats contained less elaidic acid than their pair-fed controls.

The implications of the results for the lipid metabolism of tumor-bearing rats will be discussed.

THE SYNTHESIS OF 3,4-BENZPYRENE-5-C^{14} AND A PRELIMINARY SURVEY OF ITS DISTRIBUTION IN MICE. Charles Heidelberger, Helga S. Rieke,* and S. Marguerite Weiss.* (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Because the already large body of data on the metabolism of benzpyrene includes no information as to its breakdown products, it appeared desirable to label the compound with C^{14}. The use of the tagged carcinogen will make possible more detailed studies than have thus far been carried out and will enable us to make definite comparisons with the metabolism of dibenzanthracene-9,10-C^{41}, now under investigation in these laboratories.

The synthesis was carried out by modifications of the method of Hershberg and Fieser. Phenyl magnesium bromide was treated with 7 mc. of $C^{14}O_2$ to give benzoic acid-carboxyl-C^{14} (94 per cent), which was then converted into benzoyl chloride (87 per cent). This compound was treated with perinaphthane in the presence of aluminum chloride to give benzoylperinaphthane-carbonyl-C^{14} (89 per cent). Ring closure with aluminum chloride followed by zinc dust distillation gave crude yellow benzpyrene-5-C^{14} (42 per cent), which was purified by extensive chromatography on alumina (70 per cent). The over-all yield, based on $BaCO_3$, was 22 per cent. The specific activity was 2.8 μc. per milligram.

Numerical data will be presented on the distribution of radioactivity following intravenous injection into mice, the rate of elimination from the site of subcutaneous injection, and the retention following a single painting on the skin.

THE RATE OF FECAL ELIMINATION OF RADIOACTIVITY FOLLOWING A SINGLE APPLICATION OF 1,2,5,6-DIBENZANTHRACENE-9,10-C^{14} TO THE SKIN OF MICE. Charles Heidelberger and Walter G. Wiest.* (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The absorption of a carcinogenic hydrocarbon through the skin of mice and its subsequent elimination have not been heretofore demonstrated unequivocally. We have now been able to show the elimination of radioactivity in the feces of mice following a single application of 1,2,5,6-dibenzanthracene-9,10-C^{14} to the skin. The animals were kept in cages that prevented oral ingestion or physical contact of the feces with the hydrocarbon, but which allowed sufficient movement to permit access to food and water. The feces were collected daily from individual mice and were dried and analyzed for radioactivity. Pooled samples of feces were fractionated chemically, as were pooled samples of skin. These fractionations of the feces revealed that the products of metabolism resemble those found following other modes of administration of the carcinogen, and 4 per cent of the radioactivity in the feces could be accounted for as unchanged dibenzanthracene. Analysis of the painted area showed that extensive metabolism occurred in the epidermis, where considerable amounts of 5-hydroxy-1,2-naphthalic acid were demonstrated. In addition, some protein-bound radioactivity was observed. The effect of reduced caloric intake on the fecal excretion of radioactivity was also investigated.

OBSERVATIONS ON THE NEOPLASTIC POTENTIALITIES OF MOUSE EMBRYONIC SKIN. Robert C. Hickey,* George W. Iwen,* and Nathan A. Womack. (Department of General Surgery, State University of Iowa, Iowa City, Iowa.)

The neoplastic potentialities of mouse embryonic tissue were investigated. An emulsion of lard-methylcholanthrene-embryonic skin was injected into the muscles of one thigh of 48 mice of an inbred Swiss strain; a control injection of lard-methylcholanthrene was made into the other thigh of the same animal. Equal amounts of methylcholanthrene, 0.1 mg., were used. The embryonic skin was a fresh saline minced preparation from embryos 15 and 16 days old. The observation period was 9 months. There was a distinct increase in the tumor formation on the side treated with lard-methylcholanthrene-embryonic skin emulsion.

The histologic pattern was such as to suggest tumors of mesodermal origin, most likely from the host tissues.

INFLUENCE OF CHOLINE ON THE INCIDENCE OF CARBON TETRACHLORIDE HEPATOMAS IN C3H MICE. Benjamin Highman* and Allen B. Eschenbrenner. (National Institute of Arthritis and Metabolic Diseases, and the National Cancer Institute, Bethesda, Md.)

Several series of C3H mice received 0.5 or 2 per cent CCl_4 in olive oil, 0.005 cc/gm body weight, twice weekly by stomach tube, beginning at 4–6 months of age. In the first experiment, 50 of 100 male and female mice fed a Purina Dog Chow stock diet received 1 per cent choline chloride in the drinking water, commencing 2 weeks before treatment with 2 per cent CCl_4, for 126 days. No significant difference was noted in the incidence and gross and microscopic appearance of hepatomas in the two groups. In a second experiment, 50 mice were fed a stock diet and 80 a choline-deficient diet (No. 1926 described elsewhere) beginning 2 weeks before treatment with 0.5 per cent CCl_4. After treatment for 143–185 days, hepatomas were found in 12 of 45 survivors receiving the stock diet, but in none of 18 fed diet No. 1926. In a third experiment, one group of male mice received a stock diet, one received diet 1926, and a third group received diet 1926 supplemented with 0.2 per cent choline chloride in the drinking water, beginning 2 weeks before treatment with 2 per cent CCl_4 for 138 days. Hepatomas were found in 7 of 15 survivors fed the stock diet, but in none of 23 survivors in the 2 other groups. These findings suggest that choline is not a major factor in determining the incidence of CCl_4 hepatomas in C3H mice.

AN EVALUATION OF FOUR CANCER SERODIAGNOSTIC TESTS. J. H. Hill,* R. E. Stowell, and D. J. Mulford.* (University of Kansas Medical Center, Kansas City, Kan.)

The plasma heat coagulation, methylene blue reduction, Roffo neutral red, and Munro protective colloid tests for cancer were carefully evaluated on 186 normal people, 228 patients with non-neoplastic diseases, and 140 patients with cancer. Since there was an overlapping of the results of all groups for each test, the arbitrary end-point for diagnosis of cancer was selected as that which gave only 5 per cent false positive results on healthy, fasted people—a percentage of false positives which has been suggested as reasonable for a serodiagnostic test for cancer. Under these conditions, 24–36 per cent of the patients with non-neoplastic disease had positive results for cancer with the four tests, and 32–45 per cent of the cancer patients had positive tests. By a similar comparison, the blood sedimentation rate was positive in 26 per cent of the patients with non-neoplastic diseases and in 44 per cent of the cancer patients. The mechanism of these tests was studied by observations on artificially constituted plasma prepared from blood fractions by the low temperature-ethanol method of Cohn. The results of these tests are influenced by physical and chemical characteristics of the plasma such as pH, oxygen content, and plasma proteins, which are not evident cancer-specific properties.

INHIBITION OF CHEMICAL CARCINOGENESIS IN THE SKIN OF MICE. Willard T. Hill,* William B. Wartman, Byron Riegel, Anthony Pizzo,* Philippe Shubik, and D. Warren Stanger.* (Departments of Pathology and Chemistry, Northwestern University, Chicago and Evanston, Ill.)

A number of compounds, the majority closely related to the carcinogen 9,10-dimethyl-1,2,-benzanthracene, have been tested for inhibitory action on carcinogenesis by this hydrocarbon. A mixture of the carcinogen and the inhibitory substance is dissolved in mineral oil and applied twice weekly to the skin of CAF_1 hybrid mice, the concentration of the inhibitor being that of the carcinogen.

In experiments which have lasted 31 weeks, the addition of 6,8-dimethyl-1,2-benzanthracene has prolonged the mean latent period of tumor production by 11 weeks beyond that resulting from application of a solution of 9,10-dimethyl-1,2-benzanthracene alone. Addition of 1,2,5,6-dibenzofluorene has prolonged the mean latent period by 12 weeks.

In experiments which have lasted 27 weeks, prolongation of mean latent period by approximately 8 weeks has resulted from the addition of 1,2-benzanthracene, 2-methyl-1,2-benzanthracene, 7-methyl-1,2-benzanthracene, and 8-methyl-1,2-benzanthracene.

There is evidence that some of these compounds cause a reduction in total tumor incidence in addition to a prolongation of latent period.

ENZYMATIC DEAMINATION OF 8-AZAGUANINE BY NORMAL AND NEOPLASTIC TISSUES. Erich Hirschberg,* Alfred Gellhorn, and Jacob Kream.*

(Cancer Research Institute, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

In earlier studies in this and other laboratories, it has been established that the growth of some animal tumors is strongly inhibited by 8-azaguanine, while on other neoplasms no effect can be detected. Previous studies in this laboratory on the enzymatic deamination of 8-azaguanine to the noncarcinostatic 8-azaxanthine are being continued to elucidate the reasons for this biochemical difference between tumors.

Data on the distribution of the deaminase in tissues of normal and tumor-bearing animals will be presented. The rate of deamination of azaguanine by homogenates of an azaguanine-susceptible tumor, breast adenocarcinoma 755 in C57 mice, is significantly lower than that by homogenates of the autologous livers; on the other hand, homogenates of an azaguanine-resistant tumor, Sarcoma 180 in Paris RIII mice, deaminate azaguanine at a rate comparable to that of autologous liver homogenates. This evidence suggests that the effective concentration of 8-azaguanine at the tumor sites may be enzymatically controlled.

The deaminase levels are calculated on the basis of wet and dry weights of tissue, nitrogen, DNA, and nuclear count. An analysis of the findings on these bases lends strong support to the recent claims of Davidson and Leslie, and of Price and Laird, for the significance of the DNA content or nuclear count as the fundamental unit of reference for biochemical analyses of cellular constituents.

METHYLCHOLANTHRENE AND BODY WEIGHT AS VARIABLES IN GASTRIC NEOPLASIA OF MICE.

W. F. Hollander* and L. C. Strong. (Yale University School of Medicine, New Haven, Conn.)

A test was arranged to determine whether the incidence of gastric adenomatous lesions in mice derived from the NHO stock is affected by a subcutaneous injection of 1 mg. of methylcholanthrene at age 60 days. The 3 CAMG inbred sub-strain of Br-S was used; it differs from the original Br-S, which is chocolate in color, by having a white frontal blaze and belly streak and light ("buff") belly. Three groups of about 100 mice each were set apart as nonbreeders. One of these was injected; as the mice developed local subcutaneous tumors, they were killed for autopsy, and controls of like sex and age in the second group were examined. The remaining mice were killed at about a year of age. The treated group showed no statistically significant difference in the incidence of gastric neoplasms from the second group. Body weights taken at autopsy showed that mice with gastric lesions were about 3.5 gm. (15 per cent) heavier, on the average, than those without the neoplasm; this is found in both sexes and is statistically significant. A body weight relation to the gastric lesion was not indicated, however, in a separate population derived from an outcross of Br-S mice.

A COMPARISON AND STATISTICAL ANALYSIS OF NEOPLASTIC AND NON-NEOPLASTIC LYMPH NODE CELL PARTICLE POPULATIONS (10–350 mμ).

Miriam S. Hoster,* Bette M. Hainer,* and Herman A. Hoster. (Division of Cancer Research, Department of Medicine, The Ohio State University College of Medicine, Columbus, Ohio.)

The present study represents the continuation of investigations described in a preliminary report read before the Association in 1949. A method designed to segregate macromolecular particles obtained from human neoplastic and non-neoplastic lymph node cells by centrifugation of ground lymph node suspended in a phosphate buffer-heparin mixture was described.

Polydispersed neoplastic and non-neoplastic, osmium-vapor-stained particles (10–350 mμ), obtained in high gravity centrifugation sediments (25,000 *g*), are photographed in the electron microscope at a magnification of 10,000, and the photographic plates (negatives) are projected at a magnification of 100,000. The particles are measured and histograms representing the population distribution of particles in each size range are made.

To date, histograms of 25 Hodgkin's, 5 neoplastic other than Hodgkin's, and 20 non-neoplastic lymph node cell particles have been prepared. The data recorded in the histograms of the three groups have been evaluated statistically (i.e., determination of the mean, mode, standard deviation, and curve skewness). A discussion of the adequacy of the centrifugation and particle counting technics in terms of reproducibility and statistical validity is included.

EXPERIMENTAL STUDIES IN METAL CARCINOGENESIS. I. NICKEL SARCOMAS IN RATS.

W. C. Hueper. (National Cancer Institute, Bethesda, Md.)

The rapidly increasing importance of metals and metal compounds as carcinogenic agents in man and experimental animals has provided the basis

for an extensive series of experiments on metal carcinogenesis. Of the various metals and metal compounds (chromium, chromite ore, nickel, beryllium, arsenic, uranium, asbestos) used, only parenterally injected nickel powder has produced so far a significant number of cancerous reactions at the sites of deposition (femoral cavity, pleural cavity, nasal sinuses). Of the different species employed, rats alone have shown such responses. (None have been observed up to the present time in dogs, rabbits, and guinea pigs.) Twenty-five rats received injections in the femoral cavity of 0.05 cc. of a 25 per cent suspension by volume in lanolin. Another set of 25 rats received five injections of 0.05 cc. of the same suspension in the right pleural cavity, while 20 rats were given two injections of 0.1 cc. of a 12.5 per cent suspension by volume in lanolin in the nasal sinuses. Of the total number of 70 rats, 15 died without neoplastic reactions within the first 6 months of the experiment. Of the 24 rats that died within 7–14 months after the start of the experiment, 10 showed sarcomatous growths of spindle-cell, round-cell, and giant-cell structure at the site of injection, usually originating from a markedly hyperplastic periosteal tissue. There were metastases present in several cases.

SERUM PROTEINS AS ACTIVITY INDICATORS IN CANCER. C. Huggins and Roger Baker.* (Nathan Goldblatt Memorial Hospital for Neoplastic Diseases of the University of Chicago, Chicago, Ill.)

Determination of the least thermocoagulable percentage of serum provides a simple and reproducible method of characterizing the activity of human cancer via the integrity of certain physiologic mechanisms; it has validity and usefulness. An increased percentage always indicates a deterioration of these systemic factors and a decrease reflects improvement.

17-KETOSTEROID EXCRETION IN MICE AT 5–18 MONTHS AFTER OVARIECTOMY. Frances D. Humm* (introduced by Edwin A. Lawrence). (Department of Surgery, Indiana University Medical Center, Indianapolis, Ind.; and the Department of Pharmacology, Yale University School of Medicine, New Haven, Conn.)

The 24-hour 17-ketosteroid excretion of individual mice was used as an index of adrenal cortical function. Urinary excretion levels were determined in individual Swiss mice and in hybrids of C3H and Swiss mice, at intervals of 5–18 months after ovariectomy. A total of 98 mice was tested at 5 months, 55 mice at 12–13 months, and 76 mice at 16–18 months after operation.

These animals, both hybrids and Swiss mice, fell roughly into two groups: One group showed no significant increase in 17-ketosteroid output over that found in normal litter-mate controls; the other group had a marked increase in ketosteroid output, which rose to the highest level at 12–13 months after operation, with a mean of 160 μg. and a range of 56–514 μg. The normal controls had a mean output of 81 μg., with a range of 37–166 μg. All results are expressed in μg/100 gm/24 hours.

The animals were killed and examined at autopsy at 18 months, and the adrenals fixed for histological study. Varying amounts of hyperplasia were visible grossly. A histological study is in progress.

These studies suggest that the compensatory hypertrophy of the adrenal cortex, observed by other workers in ovariectomized mice, is accompanied by functional changes which are progressive in nature. Presumably this response is mediated through the pituitary gland.

DIFFERENCES IN ADRENAL RESPONSIVENESS TO POST-CASTRATIONAL ALTERATION, AS EVIDENCED BY TRANSPLANTED ADRENAL TISSUE. Robert A. Huseby and John J. Bittner. (Division of Cancer Biology of the Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

By transplanting the adrenals of mice of two inbred strains into the common environment of adrenalectomized, gonadectomized F_1 hybrid animals, it should be possible to determine whether the differences in the response of the adrenals of the inbred strains of mice following castration are due mainly to differences in the stimulating forces acting upon the adrenals or to differences in the responsiveness of the adrenal tissue itself.

When the adrenals of A strain females (which show only minimal post-castrational adrenal changes) were transplanted to ovariectomized, adrenalectomized females of the AZF_1 hybrid cross, only one of twelve pairs of transplants became hyperplastic, while twelve of fourteen pairs of Z adrenals and six of ten pairs of AZF_1 hybrid adrenals transplanted to similarly prepared AZF_1 hybrid animals became hyperplastic and produced detectable amounts of estrogen. Similarly, Z adrenals transplanted to adrenalectomized, ovariectomized $ZCeF_1$ hybrid mice exhibited only hyperplastic changes, while Ce and $ZCeF_1$ hybrid adrenals transplanted to similar $ZCeF_1$ hybrid animals tended to form adrenal carcinomas.

These experiments would suggest that the presence or absence of post-castrational adrenal changes, as well as the histological pattern of such changes, is, at least to a large measure, a function of a genetically controlled adrenal responsiveness. Some evidence was obtained that in male mice bearing transplanted adrenals there exists some difference in the degree of stimulation that the adrenals of different strains of mice are subjected to after castration, but these differences appeared to alter only the magnitude rather than the character of the adrenal response noted.

THE CYTOTOXIC EFFECT *IN VITRO* OF ANTISERUM UPON HETEROLOGOUS MOUSE MAMMARY CANCER CELLS. David T. Imagawa,* Jerome T. Syverton, and John J. Bittner. (Department of Bacteriology and Immunology, and The Division of Cancer Biology, University of Minnesota, Minneapolis, Minn.)

Previous studies have established (*a*) that cytotoxic antibodies for cancer cells can be produced in rabbits, chickens, and guinea pigs, (*b*) that a nonspecific, heat-labile, inhibitory factor found in normal rabbit serum is absent from normal guinea pig and chicken sera, and (*c*) that antiserum representative of the mammary tumor virus exerted a marked inhibitory effect upon homologous cancer cells, whereas the same effect was slightly present in antiserum induced by tissue without the agent. The present study makes it known that the specific cytotoxic effect is independent of the strain of mouse and inseparably related, but not necessarily dependent, on the presence of the mammary tumor virus.

Cells derived from mammary cancers in mice of strain C3H were tested with samples of antiserum which had been prepared by utilizing as the antigen tissues derived from A strain mice. Conversely, cancer cells derived from mice, strain A, were tested with antiserum which had been prepared in guinea pigs by utilizing as the antigen cancerous tissues from C3H mice. The results of these experiments show (*a*) that normal guinea pig serum was not toxic to mammary cancer cells whether derived from A or C3H strains, (*b*) that antiserum which had been produced by utilizing as antigen tumor tissue of C3H mice was lethal for cancer cells of A strain, (*c*) that antiserum representative of tissues containing the tumor agent from A strain was cytotoxic for cancer cells of C3H stock, and (*d*) that antiserum representative of tissues lacking the tumor agent was not toxic for cancer cells of C3H stock.

THE METABOLISM OF β-2-THIENYLALANINE IN NORMAL AND NEOPLASTIC CELLS *IN VITRO*. John A. Jacquez, Ralph K. Barclay,* and C. Chester Stock. (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Tissue cultures of C57 black mouse heart and of sarcoma T-241 were treated with various amounts of β-2-thienylalanine. Both were severely damaged at levels of 1,000–2,000 μg/ml. This damage could be prevented by L-phenylalanine; the inhibition ratios were 10–20 M DL β-2-thienylalanine per mole L-phenylalanine (the end-point was taken as damage equivalent to about the middle of the toxicity range of β-2-thienylalanine). Tyrosine, tryptophan, leucine, isoleucine, alanine, threonine, phenylacetic acid, phenyllactic acid, and dihydroxyphenylalanine did not prevent the toxicity of thienylalanine. This is in marked contrast to the results with *E. coli* and *S. cerevisiae*, where a few amino acids besides phenylalanine can prevent the effect of thienylalanine.

In the heart cultures treated with β-2-thienylalanine, a faint reddish color appeared in the implants. This did not occur with the T-241 cultures. Addition of pyruvic, α-ketoglutaric, or phenylpyruvic acid markedly increased the production of red color in the heart cultures but not in the T-241 cultures. Furthermore, phenylpyruvic acid was almost as effective as phenylalanine in preventing the toxicity of thienylalanine with heart but not with T-241; the other two α-keto acids did not block thienylalanine in heart or T-241.

Evidence is presented that heart muscle contains a transaminase, which can transaminate between α-keto acids and β-2-thienylalanine, and that T-241 and a number of other sarcomas lack this enzyme.

HISTOCHEMICAL STUDIES ON THE BLOOD OF PLATYFISH, SWORDTAIL, AND THEIR HYBRIDS. Sophie Jakowska, Ross F. Nigrelli, and Myron Gordon. (College of Mount Saint Vincent, New York, and New York Aquarium, New York Zoölogical Society, New York, N.Y.)

Histochemical studies on blood smears and imprints of hemopoietic organs (liver, spleen, and kidney) of platyfish, swordtails, and their hybrids showed striking similarities to mammalian material. The blood elements in these fish were identified from Wright's stained preparations. Feulgen and methyl green methods were used for the localization of desoxypentosenucleic acid, Sudan

black B for lipids, and azo dye technics for the determination of alkaline phosphatase, acid phosphatase, and nonspecific esterase. The nuclei of the erythrocytes responded more intensely to Feulgen and to methyl green than the nuclei of other blood elements. The granules of the granulocytes were sudanophilic, while the erythrocytes were only tinged with this dye. The significance of this reaction in the red cell is not known, but it differs from that in the erythrocytes of the amphibian, *Triturus viridescens*, where definite perinuclear sudanophilic bodies were found. The erythrocytes in the fish studied were negative for the phosphatases, while the granulocytes gave a positive reaction. Certain tissue cells of the hemopoietic organs gave positive reactions for the phosphatases and for nonspecific esterase. The blood elements, however, appeared to be negative for the nonspecific esterase. The reactions for the phosphatases with the azo dye technics agree with results obtained by the Gormori method on mammalian blood cells.

There were no apparent differences in the tests used between normal and melanoma-bearing siblings of platyfish-swordtail hybrids.

PHOSPHORUS METABOLISM IN REGENERATING LIVER. Ralph M. Johnson,* S. Albert, and Ruth Hoste.* (Richard Cohn Radiobiology Laboratory, Detroit Institute of Cancer Research, Detroit, Mich.)

The concentration and turnover of phosphorus in certain phosphorus-containing fractions of liver in different stages of regeneration were investigated. Twenty-three rats were injected subcutaneously with radioactive phosphorus at various times following partial hepatectomy. Four hours later the animals were sacrificed. Total phosphorus determinations and radioactivity assays were made on the acid-soluble, lipid, phosphoprotein, desoxypentosenucleic acid and pentosenucleic acid fractions of the livers. The extent of mitotic activity in all tissues was estimated.

Following partial hepatectomy there were immediate increases in acid-soluble, pentosenucleic acid, phosphorus, and desoxypentosenucleic acid, and a decrease in lipid phosphorus. There was a variable increase in the amount of mitotic activity, reaching maxima on the third and seventh days. There was a parallel fluctuation in the amount of mitotic activity.

The incorporation of P^{32} in all fractions of regenerating livers was greater than that in controls; the greatest increases appeared within 24 hours following partial hepatectomy. Subsequently, P^{32} gradually decreased, approaching the values found in controls. Conversely, mitotic activity was relatively low on the first day, increased for 3 days, and thereafter fluctuated.

The results suggest that following partial hepatectomy there is an early increase in the turnover of phosphorus which takes place before any appreciable increase in the rate of cell renewal. Active cell division apparently occurs after the rate of incorporation of radioactive phosphorus passes its peak.

THE MORPHOLOGY OF HYPERPLASTIC NODULES IN THE MAMMARY GLANDS OF C3H AND C3Hb MICE. E. Elizabeth Jones. (Departments of Zoology and Physiology, Wellesley College, Wellesley, Mass.)

Within the last 2 years, 23 C3H female mice in the breeding colony maintained in this laboratory have developed mammary tumors at 15–22.5 months of age. Numerous hyperplastic nodules have been noted in their mammary glands. A study of these nodules has been made for comparison with those in C3H females which developed tumors at 5–8 and 11–12 months of age and with those in C3Hb females.

At all ages, wide variation occurred in both appearance and distribution of nodules. Characteristic changes appear with aging. In older mice, some nodules closely resemble those found in C3Hb females of comparable age.

Glands from two sublines of C3Hb mice with different incidence of spontaneous tumors are likewise found to contain hyperplastic nodules. In both sublines, many nodules are found in the glands of females 15 months of age and older. In the subline with a low tumor incidence, however, very few were found in a series of ten females examined at 12–13 months of age. The nodules in C3Hb mice sometimes closely resemble those considered "typical" in C3H females. More frequently, the acini are larger and more irregular and have a cystic appearance. Some are adenomatous. Concretions are often present in the ducts.

The frequency with which hyperplastic nodules occur in mice freed of the mammary tumor inciter raises a question as to the role of the inciter in their production.

THE EFFECT OF THE INJECTION OF NONMOUSE SUBSTANCES ON THE GROWTH OF TUMOR HOMOIOTRANSPLANTS IN MICE. Nathan Kaliss and Paulo R. F. Borges.* (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

It has been found that under certain conditions pre-injections of lyophilized normal and neoplastic

mouse tissues lead to successful "takes" of mouse tumor homoiotransplants in strains of mice in which they would normally regress (Kaliss, N., and Snell, G. D., *Cancer Research*, **11**:122, 1951). To investigate whether the effect is specific to lyophilized *mouse* tissues, parallel experiments were run with groups of C57 black mice and C57 brown-cd mice which were injected intraperitoneally either with trypan blue or with lyophilized normal tissues from rats, hamsters, guinea pigs, and rabbits. Control groups received intraperitoneal injections of either saline or lyophilized mouse tumor tissue from the strain A adenocarcinoma 15091a. Subcutaneous grafts of fresh tumors were then made into all the mice.

The following numbers, with prior injections as indicated, died with positive tumor growth: tumor, 59 out of 71 (83 per cent); trypan blue plus tumor, 39/40 (97 per cent); saline, 2/111; trypan blue alone, 1/40; rat liver, kidney, or spleen, 0/46; hamster liver, kidney, or spleen, 0/44; guinea pig liver, 0/83; guinea pig kidney, 3/68; guinea pig spleen, 1/6; rabbit serum, 0/39. It is apparent that neither trypan blue nor any of the nonmouse tissues were effective in breaking down the host's resistance to tumor homoiografts. The number of positives obtained with lyophilized guinea pig kidney does not differ significantly from the saline controls. However, there may be a synergistic effect between the lyophilized tumor and trypan blue.

THE EFFECT OF INJECTIONS OF LYOPHILIZED TUMOR TISSUE ON WOUND HEALING IN MICE.

NATHAN KALISS, DAVID M. SPAIN,* and NORMAN MOLOMUT.* (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me., Department of Laboratories and Research of Westchester County, Valhalla, N.Y., and the Waldemar Medical Research Foundation, Brooklyn, N.Y.)

It has been found that preinjections of lyophilized normal or neoplastic mouse tissues lead to a break-down of resistance to tumor homoiotransplants in inbred mice, in which the grafts would normally regress. Furthermore, in at least one instance, large doses of lyophilized tumor were followed by a break-down of resistance, while very small doses led to enhanced resistance to tumor homoiotransplants.

In the present instance it was felt desirable to compare the effects of such injections on a normal growth process, as exemplified by wound healing. Injections of a lyophilized mammary carcinoma (indigenous to the C57 black strain of mice), with total amounts injected per animal ranging from 0.0005 mg. to 50 mg., were made into mice of the C3H Jax and C57 black strains. Control groups of both strains received no injections. Following standard surgical technics, skin wounds were made with an instrument that produces two circular holes of uniform diameter (about 0.5 cm.). The skin and subcutaneous tissues were excised down to the fascial plane of the dorsal muscles Each mouse then was injected with crystalline penicillin to minimize post-operative infection. On the sixth day after wounding, the mice were sacrificed and the wounds excised for microscopic study. Fixed and stained sections were examined for relative amount of cellularity, vascularization of the granulation tissue, and degree of epithelialization. There were no significant differences among the various experimental and control groups.

FURTHER OBSERVATIONS ON INHIBITION OF LYMPHOID TUMOR DEVELOPMENT BY SHIELDING AND PARTIAL-BODY IRRADIATION OF MICE.

HENRY S. KAPLAN. (Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.)

It has been reported (Kaplan, H. S., *J. Nat. Cancer Inst.*, **10**:267, 1949) that irradiation localized over the upper or lower half of the body elicits few lymphoid tumors, in contrast to the effect of whole-body irradiation. This phenomenon has been further studied in three related experiments.

Experiment I.—One group of mice was given four doses of 300 r each at 2-week intervals over the upper half of the body only; a second group was given the same dose alternately over the upper and lower halves of the body; a third received four similarly timed treatments of 150 r each over the whole body; the fourth group was treated as the third, except that the mediastinal area was shielded with lead; and a fifth group was kept as an unirradiated control. Lymphoid tumor incidence after 16 months was, respectively, 5, 5, 52, 0, and 0 per cent.

Experiment II.—Fractionated alternate irradiation of the upper and lower halves of the body at intervals of 0, 1, 6, and 24 hours yielded lymphoid tumors in 64, 66, 48, and 51 per cent of the respective groups. This response was almost identical with that of mice receiving equivalent whole-body irradiation. Only 1 of 57 mice (2 per cent) receiving alternate half-body treatments at 4-day intervals developed a lymphoma (but through error the total dose for this group was less).

Experiment III.—This experiment was intended, in part, to study the effect of preserving ovarian function. The ovaries were transplanted to

the muscle of the right hind leg in Groups I and II, and left intact in Group III. During irradiation, mice of Group I were unshielded, while those of Groups II and III had a lead shield placed over the right hind leg. Lymphoma incidence to date has been 42, 10, and 8 per cent, respectively.

The implications of these results will be discussed and a tentative working hypothesis formulated.

EFFECT ON LYMPHOID TUMOR INCIDENCE OF CHANGES IN TOTAL DOSE, FRACTIONATION, AND PERIODICITY OF WHOLE-BODY ROENTGEN IRRADIATION. Henry S. Kaplan and Mary B. Brown.* (Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.)

A total of 1,733 black mice of both sexes was assigned by randomization of litters to 32 groups, all of which were irradiated at 33 ± 3 days of age. Successive groups received systematic increments of total dose, administered either as a single treatment or in 2, 4, or 8 equal daily fractions. Equivalent total doses were also given in fractionated treatments at intervals of 4, 8, and 16 days. Animals were observed for: (*a*) initial radiation mortality and (*b*) the development of lymphoid tumors.

Lymphoma incidence roughly paralleled total dose, within the range investigated, for either a single exposure or fractionated exposures at daily intervals. Response to equal total doses was not modified by the degree of fractionation. For a given total dose, however, there was a striking increase in incidence when an interval of 4–8 days occurred between treatments. Response was no greater than for daily treatments when the interval was increased to 16 days.

In contrast, mice exhibited a pronounced ability to recover from the lethal effect of a given total dose with either increasing fractionation or, in particular, the interposition of an increasing interval between treatments. The difference in dose-response characteristics indicates that roentgen radiation operates through different mechanisms in eliciting lethal and leukemogenic responses in mice.

INHIBITION BY TESTOSTERONE OF RADIATION-INDUCED LYMPHOID TUMOR DEVELOPMENT IN INTACT AND CASTRATE MALE MICE. Henry S. Kaplan and Mary B. Brown.* (Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.)

Strain C57 black male mice were distributed as litter mates among four groups of about 50 mice each. Two groups were maintained intact, the other two castrated at 2 months of age. Whole-body irradiation was started 3–4 days later; all groups received a total of 675 r in 4 equal fractions at 4-day intervals. One castrate and one intact group received intramuscular injections of testosterone (25 mg/cc in 0.35 per cent aluminum phosphate suspension) starting on the first day of irradiation, while the other two groups were injected with the aluminum phosphate suspension (placebo) alone. The injection volume was 0.02 cc. of either material given twice weekly for 10 weeks.

To date, lymphoid tumors have been observed in only 3 of 49 intact, testosterone-treated animals (6 per cent), and in 4 of 50 castrate, testosterone-treated animals (8 per cent), as contrasted with an incidence of 37/52 (71 per cent) and 43/52 (83 per cent) among intact and castrate placebo-treated mice, respectively. Thus, testosterone exerts a striking inhibitory effect on the development of lymphoid tumors in irradiated male mice, confirming the earlier observations of Murphy (*Cancer Research*, **4**:622, 1944) on spontaneous lymphomas in Rockefeller strain mice, and of Gardner (*Proc. Soc. Exper. Biol. & Med.*, **75**:434, 1950) on radiation-induced lymphomas in female BC mice. Under the conditions of the present experiment, castration of mature male mice did not significantly modify lymphoid tumor incidence.

ADRENAL CORTICAL FUNCTION AND LYMPHOID TUMOR INCIDENCE IN IRRADIATED MICE. Henry S. Kaplan, Mary B. Brown,* and Sumner N. Marder. (Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.)

In earlier experiments, desoxycorticosterone acetate and Lipo-adrenal cortex extract failed to modify lymphoid tumor incidence in irradiated mice (Kaplan, H. S., *Acta Union international. contre le cancer* [to be published]). Two recent experiments reveal, however, that cortisone significantly inhibits and adrenalectomy enhances the leukemogenic effect of irradiation.

Experiment I.—Groups of C57 black mice received local or whole-body irradiation and/or cortisone in various combinations. In the dose administered, whole-body irradiation yielded lymphomas in 41 of 50 mice (82 per cent). When cortisone injections were started simultaneously with irradiation, only 18 of 48 mice (37 per cent) developed these tumors, and when cortisone treatment was deferred until 6 weeks after irradiation, only 6 of 23 animals (26 per cent) responded. Cortisone

alone yielded no tumors. Irradiation over the mediastinum alone, or over the rest of the body with the mediastinum shielded, failed to elicit lymphomas whether or not cortisone was also given.

Experiment II.—Two groups of C57 black mice were adrenalectomized and a third subjected to sham operation. Group I received no further treatment; the other adrenalectomized group and the sham-operated group received fractionated whole-body irradiation in four treatments at 2-week intervals. Initial mortality was 20, 67, and 3 per cent, respectively. Lymphoid tumors occurred among 0 of 24 (0 per cent), 13 of 20 (65 per cent), and 12 of 59 surviving mice (20 per cent), respectively, indicating a significant increase in susceptibility to lymphoma induction among adrenalectomized mice.

THE EFFECT OF CORTISONE ON THE GROWTH OF TUMORS EXPLANTED TO THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO.

D. A. Karnofsky and L. P. Ridgway.* (Division of Experimental Chemotherapy, Sloan-Kettering Institute, New York, N.Y.)

The effect of various agents on tumor growth is being studied in the fertile hen's egg by means of chorioallantoic explants of chick and mouse tumors. The Rous sarcoma and fowl leukosis Strain RPL-12 (RPL-12 was obtained through the courtesy of George E. Cottrall, Regional Poultry Research Laboratory, East Lansing, Mich.) were the chicken tumors used. Explantation of the Rous sarcoma resulted in a large tumor at the site of implantation and a generalized hemorrhagic disease of the embryo, particularly conspicuous in the liver. The 8-day embryo usually died 6–9 days after explantation, owing to a severe hemorrhage. RPL-12 explants also produced a large nodule on the chorioallantoic membrane and extended to involve the liver and spleen. These embryos also died within 8 days after explantation. The mouse tumors used in this study were Sarcoma 180, osteogenic sarcoma (Ridgway), mammary tumor E 0771 and C1300. These tumors grew rapidly and did not produce any gross changes in the embryo.

Cortisone acetate, at a single dose of 1 mg. per egg, applied to the chorioallantoic membrane of the 8-day embryo, produced a marked inhibition in the growth of the chick embryo and characteristic changes in development; 3 mg. per egg injected into the yolk sac had a similar effect. The effect of these doses on the explanted tumors was determined by measuring their rate of growth in the egg, the extension of the neoplastic cells to the embryo (in the case of the chicken tumors), their histological appearance, and the viability of the tumor when assayed into susceptible animals. In general, cortisone decreased but did not completely inhibit the growth of the tumors studied.

FATE AND DISTRIBUTION OF PODOPHYLLOTOXIN IN ANIMALS.

M. G. Kelly,* J. Leiter, A. R. Bourke,* and P. K. Smith. (National Cancer Institute, Bethesda, Md., and the Department of Pharmacology, The George Washington Medical School, Washington, D. C.)

A bioassay method, based on the lethality of podophyllotoxin to the chick embryo, has been developed and applied to the study of the disposition of podophyllotoxin by the intact animal and of the action of animal tissues on the drug *in vitro*.

The material prepared by acetone extraction of carcasses of rats and mice killed at intervals after injection with podophyllotoxin was inoculated into the yolk sac of chick embryos. The results suggested that the drug had been metabolized by the intact animal within 1 hour to a product nonlethal to the chick embryo or insoluble in acetone. There was no evidence of accumulation or retention of the unaltered drug in liver, kidney, spleen, blood, or tumor tissue.

Incubation of podophyllotoxin with homogenates of carcass, kidney, spleen, muscle, intestine, liver, blood serum, red blood cells, and tumor tissue of untreated rats and mice did not appear to destroy or alter the podophyllotoxin in 4 hours.

By this method, no evidence of excretion of unaltered podophyllotoxin was obtained in rats and dogs.

Variations in the toxic effect on the chick embryo of such extracts were paralleled by analogous variations in their potency to damage Sarcoma 37

EFFECT OF PODOPHYLLOTOXIN ON HEMATOPOIETIC SYSTEM OF ADRENALECTOMIZED RATS.

M. G. Kelly,* J. Leiter, R. C. MacCardle, and P. K. Smith. (National Cancer Institute, Bethesda, Md., and the Department of Pharmacology, The George Washington University Medical School, Washington, D.C.)

Podophyllotoxin had been reported previously to produce histologic damage to the bone marrow and adrenal cortex and transitory leukopenia (including eosinopenia), followed by a leukocytosis in 48–72 hours.

To determine the influence of the adrenal gland on the hematopoietic response, a study was made of the blood changes in adrenalectomized rats

treated with a single intraperitoneal injection of toxic doses of podophyllotoxin. Within 1 hour there was a significant leukopenia (mononuclear, polymorphonuclear, eosinophilic); within 24 hours the leukocyte levels had returned to normal, and no further significant changes occurred during a 72-hour observation period.

The ratio of spleen weight to total body weight after treatment with podophyllotoxin was increased in adrenalectomized rats but was decreased in normal rats. This suggests that the spleen was involved in the leukopenia observed in adrenalectomized rats.

CHOLINE OXIDASE ACTIVITY IN RAT LIVER AND RAT LIVER TUMORS. C. J. KENSLER and H. LANGEMANN.* (Department of Pharmacology, Cornell University Medical College, New York, N.Y.)

The centrifugation method of Hogeboom, Schneider, and Pallade (*J. Biol. Chem.*, **172**:619, 1948) was used to separate rat liver into nuclear, large granule (mitochondria), and supernatant fractions. Approximately 70 per cent of the choline oxidase activity of this tissue is associated with the mitochondria fraction. Sufficient cytochrome c was retained in the mitochondria fraction to permit 50 per cent of maximal activity in the absence of added cytochrome c. Rat liver tumors produced by feeding 3′-methyl-N, N-dimethyl-*p*-aminoazobenzene contained only small amounts of choline oxidase activity (less than 10 per cent of normal liver), in agreement with the finding of Viollier (*Helv. Physiol. Acta*, **8**:C 37, 1950) that rat liver tumors produced by feeding N,N-dimethyl-*p*-aminoazobenzene (DMB) contained only small amounts of choline oxidase activity.

Dubnoff (*Arch. Biochem.*, **24**:251, 1949) has presented evidence that the methyl groups of choline are not available until choline has been oxidized by choline oxidase to betaine. Hence, any circumstances favoring decreased choline oxidase activity in hepatic tissue might be expected to produce effects in this tissue which resemble those of a partial choline deficiency, at least insofar as choline's function as a methyl donor is concerned. As hepatic neoplasms are produced by a chronic choline deficiency under certain dietary conditions (Salmon and Copeland, *Am. J. Path.*, **22**:1059, 1946) the depletion of choline oxidase activity may be of importance in the mode of action of the azo dye carcinogens. Some support for this possibility is provided by the observation of Viollier (*Helv. Chim. Acta*, **31**:387, 1948) that choline oxidase activity is lowered by riboflavin deficiency, which was found earlier by Kensler, Sugiura, Young, Halter, and Rhoads (*Science*, **93**:308, 1941) to facilitate tumor production by DMB and by our observation that choline oxidase activity is sensitive to inhibition *in vitro* by metabolites of DMB.

INTRINSIC TISSUE RESPONSE TO INDUCTION OF PULMONARY TUMORS. ARTHUR KIRSCHBAUM and JOYCE R. SHAPIRO.* (Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn.)

The objective was to determine whether pulmonary tissue from a strain of mice resistant to the induction of tumors would retain its resistant character in a host susceptible to pulmonary tumor induction. Dba-212 mice were relatively resistant (9 per cent induced tumors with 1–3 nodules), and Bagg albino 100 per cent susceptible (10–18 nodules) to the induction of pulmonary tumors by urethan. F_1 hybrids between the two strains were 100 per cent susceptible.

Pulmonary tissue (2 × 2 mm.) of newborn dba mice (resistant) was grafted into the areolar tissue of the right ear and lung tissue from newborn Bagg albinos (susceptible) into the left ear of F_1 hybrid crosses. After the subcutaneous grafts had become vascularized, F_1 hybrids bearing them received six weekly anesthetic doses of urethan. Grafts were observed for 9–13 months. Controls grafts were made into F_1 hybrids not treated with urethan.

Pulmonary tumors appeared in twelve of seventeen Bagg albino tissue grafts, whereas only one tumor developed in seventeen dba grafts. All F_1 hybrid host lungs contained multiple urethan-induced adenomas. No tumors appeared after 10–12 months in twenty Bagg albino and twelve dba control ear grafts (hosts not injected with urethan). Histologically, tumors of grafts were identical with those of the lung *in situ*.

Thus, pulmonary tissues of mice resistant to the induction of pulmonary tumors exhibited target tissue resistance when present in hosts susceptible to the induction of pulmonary tumors. Grafted tissue of intrinsic susceptibility became tumorous under the same conditions.

TRACER STUDIES ON THE METABOLISM OF THE GARDNER LYMPHOSARCOMA. SAUL KIT* and DAVID M. GREENBERG. (Division of Biochemistry, University of California, School of Medicine, Berkeley, Calif.)

The relationship between the energetic processes of the cancer cell and protein synthesis has been investigated. The uptake of radioactive amino acids into the protein of a suspension of the

Gardner lymphosarcoma cells of the mouse has been used as an indicator of protein synthesis. A coupling between glycolysis and the uptake of radioactive glycine, alanine, leucine, and phenylalanine into the tumor cell protein was demonstrated.

The existence of the tricarboxylic acid cycle in the tumor and the importance of its role as a source of energy for protein synthesis was inferred from the following facts: The tumor cells oxidize succinic acid, and this oxidation is correlated with an increased uptake of glycine, leucine, and phenylalanine into cell protein. Krebs cycle inhibitors markedly reduce respiration and amino acid uptake. Radioactive acetate is oxidized to radioactive CO_2, and part of the radioactivity may be found in the cell protein. Fluoroacetate inhibits this process. When tumor cells are incubated with oxalacetate and acetate, citrate is formed.

The over-all protein turnover rate of the Gardner lymphosarcoma cell was compared with that of lymphocytes from the spleens of normal mice. It was shown that the rate for the tumor cell was not greater than that for the normal cell, and the implications of this on the problem of tumor metabolism are discussed.

CHOLINESTERASE AND TRIBUTYRINASE ACTIVITY OF RAT LIVER AND RAT LIVER TUMORS. H. LANGEMANN* and C. J. KENSLER. (Department of Pharmacology, Cornell University Medical College, New York, N.Y.)

Viollier and Waser (*Helv. Physiol. Acta*, **8:** C 39, 1950) have reported that the cholinesterase activity of rat liver tumors produced by azo dyes is higher than that of normal rat liver and that tributyrinase activity is decreased in these tumors. Since, in other species, tributyrinase activity and "nonspecific" cholinesterase activity have been reported to parallel one another, these results suggested that "specific" cholinesterase was selectively increased. Normal rat liver contains both types of cholinesterase (Ord and Thompson, *Biochem. J.*, **46:**346, 1950).

An examination of the cholinesterase and tributyrinase activity of rat (Wistar strain) liver and of liver tumors produced by feeding 3′-methyl-N,N-dimethyl-*p*-aminoazobenzene has shown that tributyrinase activity of the tumors uniformly was approximately 10 per cent of that of normal liver (μl CO_2/gm wet tissue/hr = 1200), whereas cholinesterase activity was, in general, though not always, increased.

Using the rates of hydrolysis of benzoylcholine and mecholyl to differentiate between "nonspecific" and "specific" cholinesterase, it was found that either or both types were increased in individual tumors. Different tumors, even from the same animal, showed widely different activity and different distribution of activity between the two types of cholinesterase. Tributyrinase activity did not parallel "nonspecific" cholinesterase astivity.

The separation of rat liver by centrifugation in 0.88 M sucrose into nuclear, large granule, and supernatant fractions has shown that about 70 per cent of both the cholinesterase and tributyrinase activity was present in the supernatant fraction.

CELL FRACTIONATION OF RAT AND MOUSE TUMORS; COMPARISON WITH NORMAL ADULT RAT LIVER, KIDNEY, AND THYMUS. ANNA KANE LAIRD* (introduced by H. P. Rusch). (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

A variety of tumors was homogenized and separated by differential centrifugation into nuclear, large granule, small granule, and supernatant fluid fractions. The tumors studied include the Jensen sarcoma, Flexner-Jobling carcinoma, Walker 256 carcinosarcoma, hepatomas induced by 3′-methyl- and 4′-fluoro-dimethylaminoazobenzene, and by acetylaminofluorene in the rat; and the Gardner lymphosarcoma, a transplantable mammary carcinoma, and an adrenal tumor in the second transplant generation in the mouse. Normal adult rat liver, kidney, and thymus were studied similarly. Nucleic acids (by Schneider's method) and protein-N were determined in each fraction obtained. A nuclear count was made on the whole homogenate before fractionation, and the quantities of nucleic acids and protein-N in each fraction were expressed as gm. $\times 10^{-12}$ per cell.

In spite of the variety of the tissues of origin of these tumors in two animal species, when the analytical values are expressed in terms of an average cell, the tumor cells are found to be so similar as to be indistinguishable from one another in the quantity of each constituent in each fraction and in the pattern of distribution of these within the cell. The average liver cell and the average kidney cell are quite different from the average tumor cell, but they resemble each other very closely in the pattern of distribution of protein-N; however, the absolute quantities per cell are less in the kidney than in the liver. The average thymus cell is similar to tumor cells in the distribution of protein-N, but the thymus cell is very much smaller than the average tumor cell.

For each cell fraction, the ratio of pentose-nucleic acid to protein-N is relatively constant

among the tumors studied. During the early stages of regeneration in the rat liver, these ratios rise from the normal values to values approaching those found in the tumors.

OBSERVATIONS ON PROPERTIES OF MAMMALIAN LEUKEMIC CELLS RESISTANT TO FOLIC ACID ANTAGONISTS. L. W. Law. (National Cancer Institute, Bethesda, Md.)

A transplantable acute lymphoid leukemia has given rise to three variants when grown in serial transfer in mice receiving the MTD (maximum tolerated dose) of three different folic acid antagonists: amethopterin, aninopterin, and adenopterin.

In the case of one of the variants, susceptibility to the specific antagonist has remained unchanged following 23 consecutive serial transfers in mice free of the antagonist. This is evidence of a stable and irreversible heritable change.

Concentrations of folic acid antagonists which completely inhibit the growth of lymphoma tissue of the susceptible subline appear to be essential for optimal growth of the variant sublines.

The resistant variants have been shown to be cross-resistant to all other folic acid antagonists tested but sensitive to several other anti-leukemic compounds: 8-azaguanine, alpha-peltatin, and triethylene melamine.

Considerations of the manner of origin of these variants and the significance of these findings to the chemotherapy of leukemia will be discussed.

THE CONTROL OF A TOXIC FACTOR IN THE RABBIT V_2 CARCINOMA. Edwin A. Lawrence, Donald E. Bowman,* and Donald B. Moore.* (Department of Surgery and the Departments of Biochemistry and Pharmacology, Indiana University Medical Center, Indianapolis, Ind.)

In an experiment that required the intravenous injection in rabbits of a suspension of V_2 carcinoma cells, it was found that many of the animals died immediately from an anaphylactoid reaction similar to that observed following the intravenous administration of trypsin. Therefore, the possible protective effect of some of the bean trypsin inhibitors, described by one of us (Bowman, D. E., *Arch. Biochem.*, **16**:109, 1948), was investigated.

The donor tumor was obtained from implants in the thigh muscles as well as in subcutaneous tissues. The tumor fragments were minced with a tissue grinder, agitated with normal saline, and a 1:10 cell suspension used in the injections. Seventy per cent of the animals injected with from 0.8 to 10 cc. of this material died immediately. Nineteen per cent of the animals developed severe immediate reactions but have survived, and 11 per cent have had no reaction. When from 2.5 to 5 mg. of the globulin trypsin inhibitor of soybeans was added to the cell suspensions, 89 per cent of the rabbits had no reactions and have survived; 7 per cent had immediate reactions and died; and 4 per cent had delayed reactions followed by death. The inhibitor has had no observed effect on the growth of the tumor. The nature of the factor causing the lethal action of the suspension and the mechanism of the protection afforded by the trypsin inhibitor are being investigated and will be discussed.

INFLUENCE OF SEX ON 2-ACETYLAMINOFLUORENE-INDUCED LIVER TUMORS IN RATS AND MICE. J. H. Leathem. (Bureau of Biological Research, Rutgers University, New Brunswick, N.J.)

2-Acetylaminofluorene, incorporated as 0.03 per cent of a fox chow diet, induced liver changes in 90 per cent of male rats (Wistar) but influenced less than 10 per cent of female rats in 6 months. Paired feeding of male rats against *ad libitum*-fed females seemed necessary to clarify the sex difference. After 6 months, average body weight was 238 gm. for female rats, and cyst formation was observed in one liver of the twelve studied. Body weight was 241 gm. for nine pair-fed male rats, and hepatomas were present in seven. Liver weight averaged 18.6 gm. in males and 11.7 gm. in females following drug feeding. Furthermore, total liver protein and the ratio of liver protein to body weight was significantly greater in male rats. Livers of 2-AAF-fed rats contained more protein than pair-fed or *ad libitum*-fed rats not receiving the carcinogen. A decrease in hematocrit and an increase in plasma globulin concentration were observed in male rats. A decrease in seminal vesicle weight and an increase in splenic weight were the only other organ changes not accountable for by body weight differences.

2-AAF as 0.1 per cent of a fox chow diet did not induce hepatomas in Swiss mice of either sex in 6 months. A semi-synthetic diet containing 24 per cent casein and 8 per cent Mazola with 0.1 per cent 2-AAF induced hepatomas in 10 of 13 males but did not influence female mice.

TRACER STUDIES OF RADIOACTIVE SODIUM ESTRONE SULFATE (S-35) IN CASES OF ADVANCED BREAST CANCER. Edward F. Lewison, Georgeanna S. Jones,* Howard W. Jones, Jr., J. Elliot Levi,* and Hannah E. Silberstein.* (De-

partments of Surgery and Gynecology, Johns Hopkins Hospital, and Departments of Surgery and Medicine, Sinai Hospital, Baltimore, Md.)

Estrogens are known to have a selective action upon the epithelium of the breast, the uterus, and the vagina. This specific action is due either to the selective sensitivity of the target end-organ, or possibly to the selective absorption by these tissues.

To investigate this selectivity, radioactive sodium estrone sulfate labeled with sulfur 35 has been used in tracer studies of estrone distribution and metabolism in both animals and man. Seven cases of advanced breast cancer have been studied in this investigation. The tagged hormone was injected subcutaneously prior to surgery, and at the time of operation multiple biopsies were performed. Radioassays have been correlated with bioassays.

It can be said that from our clinical experimental evidence when sodium estrone sulfate is administered to patients with breast cancer, the concentration of radioactivity in the tumor tissue is significantly higher than could be accounted for by random distribution throughout the body.

THE INTRACELLULAR DISTRIBUTION OF CATHEPSIN, LEUCINE AMIDASE, AND BENZOYLARGININE AMIDASE ACTIVITIES IN NORMAL RAT TISSUES AND A PRIMARY RAT HEPATOMA.

Mary E. Maver and Antoinette E. Greco.* (National Cancer Institute, Bethesda, Md.)

The fractions of the tissue homogenates were separated by differential centrifugation in 0.25 M sucrose, according to the method described by Schneider and Hogeboom (*J. Biol. Chem.*, **183:** 123, 1950). The percentage distribution of catheptic activity, as followed by the ability to hydrolyze hemoglobin, varied with the fractions of the different tissues, but the highest specific activity (per milligram N in fraction) of this proteinase was found in the mitochondrial fraction of all of the tissues.

Curves of the specific activity at different hydrogen ion concentrations showed that benzoylarginine amide was hydrolyzed at two pH optima, pH 6.2 and 4.0. At pH 6.2, the mitochondrial fraction of normal rat liver hydrolyzed over 50 per cent of the total activity of the homogenate, with a specific activity 6 times that of the homogenate. The mitochondrial fraction of the hepatoma homogenates contained 40 per cent of the total activity and 4 times the specific activity at pH 4.0. The supernatant fractions of the hepatoma homogenates had lower specific activity but hydrolyzed over 50 per cent of the total activity at pH 6.2 and at pH 4.0.

In all the tissues studied, most of the leucine amidase activity was found in the supernatant fraction of the homogenate. The supernatant fraction of the liver homogenate had a specific activity 9 times that of the homogenate.

DISTRIBUTION OF EMBOLIC TUMOR CELLS IN RELATION TO DISTRIBUTION OF RESULTING TUMORS.

Morton McCutcheon, Dale Rex Coman, and Robert P. DeLong.* (Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pa.)

The laws governing metastases of malignant tumors no longer need be regarded as mysterious or inaccessible to investigation. The long known differences in distribution of secondary tumors, their conspicuously greater frequency in adrenals and kidneys, their comparative scarceness in muscle, spleen, and thyroid, is explainable by the mechanics of circulation rather than by any peculiarities of the organs themselves. This mechanical hypothesis, obviating the "soil hypothesis," is that the number of secondary tumors arising in any organ is dependent on the number of tumor emboli that reach it.

The validity of this mechanical explanation of metastasis was tested by injecting cell suspensions of the Brown-Pearce tumor into the left cardiac ventricle of rabbits. Half the animals were immediately sacrificed, and the distribution of tumor cells (specially marked) ascertained in microscopic sections. The other rabbits were allowed to survive long enough for tumors to develop and were then sacrificed and the distribution of tumors ascertained. Kidneys, stomach, adrenals, eyes, diaphragm, pituitary, spleen, thyroid, and testes were involved in descending frequency. When listed according to number of emboli, the order was essentially the same. This result supports the mechanical hypothesis that metastatic tumors are numerically proportional to the tumor emboli that reach the various organs.

A MICROFLUOROMETRIC METHOD WITH POTENTIALITIES FOR THE SCANNING AND THE DETECTION OF CANCER CELLS IN CERVICAL SMEARS.

Robert C. Mellors, George N. Papanicolaou, and Adele Glassman.* (Sloan-Kettering Institute for Cancer Research and the

Strang Clinic, Memorial Center, New York, N.Y.)

Analytic studies which have been made of the physical and the chemical properties of intact cells include: (*a*) light absorption in the ultraviolet, the visible and the infrared regions, and (*b*) light emission in the form of primary and secondary fluorescence. A portion of the work which was undertaken with the intention of developing a quantitative scanning method for the detection of cancer cells in diagnostic smears is described herewith.

In view of the morphologic significance of nuclear hyperchromasia in certain types of cancer cells, an analytic method was developed for the determination of the relative contents of nuclear chromatin. Cells were stained under conditions which chemically favor selective combination of a basic fluorochrome with nuclear chromatin and were examined with a fluorescence microscope illuminated with a *constant* source of ultraviolet light. The relative intensities of the visible light emitted by the dyestuff bound to nuclear chromatin was measured with a photomultiplier tube.

A comparison of normal epithelial cells (class I–II: squamous, parabasal), abnormal cells (class III), and cancer cells (classes IV–V) of the cervix uteri indicated that as the cells became abnormal both the range and the average of fluorescence intensities of nuclei increased. The likelihood of a chance occurrence of the differences between the average values for the normal cells and those of classes III and IV–V was extremely remote, with a probability of less than 1 in 10,000 for the cell samples of the order of 30–50 per class.

TREATMENT OF LYMPHOMATA WITH TRIETHYLENE MELAMINE.

Leo M. Meyer, Milton R. Beyers,* Arthur Sawitsky,* Conrad Brahin,* and Norton D. Ritz.* (Bronx V. A. Hospital and the Goldwater Memorial Hospital, New York, N.Y.)

Thirty-nine patients with various lymphomatous diseases were treated orally with triethylene melamine. Dosage ranged from 2.5 mg. per day for 3 days to 10 mg. per day for 2 days. Total doses were 10–125 mg. for periods of 6–200 days. Nausea, anorexia, or vomiting was observed in 18 persons. Diarrhea was noted in 5 instances. Hemoglobin and red blood cells rose during treatment in 2 persons. Platelets appeared unaffected. Bleeding tendency was not aggravated. Transient leukopenia occurred in 19 cases. Eosinophilia varying from 3 to 22 per cent was found in 21 patients. Bone marrow became hypocellular in 15 individuals. Pancytopenia with aplasia of marrow resulted in the death of 3 subjects. Toxicity was not related to dose of the drug.

In 27 instances of lymphadenopathy, reduction of nodes was observed in 14, and no change occurred in 13. Splenomegaly in 28 patients was reduced in 16 and unchanged in 12. Hepatomegaly was decreased in 12 persons and was unaltered in 7. Among 9 patients with chronic lymphocytic leukemia, there were 2 complete remissions and 2 cases distinctly improved. Five patients failed to respond. Of 12 patients with Hodgkins disease, there were 3 long excellent and 8 short transient remissions. Of the 8 patients with chronic myelocytic leukemia, there were 3 very good reactions, 3 moderately good, and 1 poor. In 2 persons with lymphosarcoma 1 response was excellent and the other moderately good. Six of the 8 patients with acute leukemia showed only short periods of improvement.

RESPONSE TO HORMONES OF BENIGN AND MALIGNANT TUMORS OF COMMON MAMMARY ORIGIN IN THE RAT.

J. Millar* and R. L. Noble.* (Department of Medical Research, University of Western Ontario, London, Ontario, Canada.)

Extensive studies have been conducted on a transplantable mammary fibroadenoma and on sarcoma arising, therefrom, in the rat. The benign tumor showed accelerated growth under the influence of a low dosage of estrogens, crude anterior pituitary extracts, or pregnancy. Reduction in growth was found after ovariectomy or in male animals. Striking growth retardation followed treatment with a high dosage of estrogens. Malignant tumors were little affected by corresponding treatment. The characteristics of the two tumors and factors influencing the malignant transformation will be discussed.

ON THE METABOLISM OF 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE-N-METHYL-C^{14} IN THE RAT.

E. C. Miller, A. M. Plescia,* J. A. Miller, and C. Heidelberger. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Approximately 70 per cent of the C^{14} from 3′-methyl-4-dimethylaminoazobenzene-N-methyl-C^{14} was expired as carbon dioxide within 48 hours after the administration of a single dose to rats by stomach tube. Approximately 10 per cent of the activity was excreted in the urine and feces, and the remainder was found in the carcass. Of the

radioactivity left in the animal after one or several doses, 15–20 per cent was contained in the protein and 5 per cent in the choline, and the specific activity of both of these components was 2–3 times higher in the liver than in the rest of the body. Fifteen to 35 per cent of the activity in the protein was contained in the serine, and all the radioactivity in this amino acid was in the β-position. Since other investigators have shown that formate and formaldehyde are incorporated only into the β-position of serine, at least one N-methyl group from the dye is apparently oxidized through these intermediates or a related one-carbon compound. Most of the radioactivity in the choline was contained in the N-methyl groups, and, although some direct transmethylation cannot be excluded, it seems most likely that the activity was derived from formate or formaldehyde. Rats deficient in either folic acid or vitamin B_{12} incorporated much less radioactivity into the liver serine and choline than control rats supplemented with both of these vitamins.

A DIETARY FACTOR INVOLVED IN THE OXIDATIVE N-DEMETHYLATION OF 3-METHYL-4-MONOMETHYLAMINOAZOBENZENE. J. A. Miller, Raymond R. Brown,* E. C. Miller, and Gerald C. Mueller. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

With the use of the demethylation system described elsewhere in these abstracts by Mueller and Miller (p. 271), a dietary factor has been found which determines the rate at which properly fortified homogenates of rat and mouse liver oxidatively N-demethylate 3-methyl-4-monomethylaminoazobenzene. Mouse liver homogenates are about twice as active as those from rats, and the mouse responds more easily to the depletion of and repletion by this factor. The factor appears to be relatively lacking in a crude diet derived from plant and milk products. The factor is present in relatively large amounts in commercial chows containing meat and fish products. A highly purified diet adequate in all gross respects and supplemented with high levels of all of the recognized crystalline vitamins permitted no higher activity than the deficient crude diet. However, maximum activity was obtained when this purified diet was supplemented with various crude materials such as 1:20 liver extract, reticulogen, fish meal, and fish solubles. Although the dietary factor is apparently different from the known crystalline vitamins, the present data do not permit a decision as to whether the deficiency is that of a nutrient required in small amounts or that of a substrate which stimulates the formation of an adaptive enzyme.

EVIDENCE AGAINST THE PARTICIPATION OF BENZIDINE AND SEMIDINE REARRANGEMENTS IN CARCINOGENESIS BY 4-DIMETHYLAMINOAZOBENZENE. J. A. Miller, E. C. Miller, and R. W. Sapp.* (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The suggestion has been made that the hepatocarcinogenicity of 4-dimethylaminoazobenzene in the rat may depend on the benzidine rearrangement of the hydrazo derivative to form 2,4′-diamino-5-dimethylaminobiphenyl. Previous publications from this laboratory have shown that the latter compound is noncarcinogenic and that the carcinogenicity of 4-dimethylaminoazobenzene is *increased* by the substitution of fluorine in either the 4′, 3′, or 2′-positions. The same finding has now been made for the 2-fluoro derivative. The presence of the strong C-F bond in these positions should *decrease* the activity if a rearrangement of a hydrazo metabolite was involved in the carcinogenic process. More conclusive evidence against this hypothesis now exists, since 2′,4′-difluoro- and 2′,4′,6′-trifluoro-4-dimethylaminoazobenzene have also been found to be *more* carcinogenic than the unsubstituted dye. Only one of the five possible benzidine or semidine rearrangements could possibly occur in the case of the trifluoro derivative; this lone possibility involves a semidine rearrangement to the 2-position. However, the high activity of the 2-fluoro derivative makes this possibility very unlikely. Thus, while such rearrangements may occur in the metabolism of 4-dimethylaminoazobenzene, it is doubtful that they play any decisive role in carcinogenesis by this dye. Indeed, the carcinogenic activities of all these fluoro dyes indicate that of the four positions involved, i.e., 4′, 3′, 2′, and 2, probably *none* are *directly* concerned in the carcinogenic process. While the 3-position remains to be tested in this regard, the above data and the essentiality of the azo group and at least one N-methyl group for carcinogenicity indicate that the *initial* carcinogenic reaction probably involves one or both of these groups.

ENHANCEMENT OF THE ONCOLYTIC EFFECT OF THE RUSSIAN ENCEPHALITIS VIRUS. Alice E. Moore. (Sloan-Kettering Institute, New York, N.Y.)

In the study of the effect of neurotropic viruses on transplantable tumors in mice, it was found

that their oncolytic effects were so closely associated with their neurotropism that the tumor-bearing animals always died of the infection. With chicken leukosis RP12 and two of these viruses, tumor destruction is not always associated with the death of the host. Therefore, it seemed possible that by continuous passage from tumor to tumor in cancer-bearing animals one might be able to separate the neurotropic portion of the Russian encephalitis virus from its oncolytic portion.

Although it has not been possible to influence the neurotropism of the virus, the following changes have taken place: (*a*) By continuous passage in the Sarcoma 180, the oncolytic ability of the virus for this tumor has been increased. (*b*) By continuous passage in brain, the oncolytic ability of the virus has greatly decreased. (*c*) By continuous passage in a nonsusceptible tumor (Wagnar osteogenic sarcoma), the virus has been changed so that it will now destroy it.

The experimental data for these conclusions will be given.

THE USE OF THE VERTEBRAL VENOUS SYSTEM AS A PATHWAY FOR THE DISTRIBUTION OF A TRANSPLANTABLE CARCINOMA.

Donald B. Moore* and Edwin A. Lawrence. (Department of Surgery, Indiana University Medical Center, Indianapolis, Ind.)

Demonstration of the vertebral venous system in rabbits has been accomplished by cannulating a dorsal penile vein, exerting pressure on the inferior vena cava through the abdominal wall at the level of the lower lumbar vertebrae, injecting colloidal thorium dioxide, and recording the distribution of the thorium dioxide by roentgenograms of the whole body of the animal. The same distribution was obtained more easily and satisfactorily by operatively ligating and dividing the inferior vena cava as well as the superficial inferior epigastric vessels and injecting thorotrast into a femoral vein. A volume of 11 cc. of thorotrast produced excellent filling of the entire vertebral venous system and a large number of its tributary veins.

With the same technics of vessel ligation and thorotrast injection, cell suspensions in saline of the rabbit V_2 carcinoma were injected into the femoral veins. Paraplegia and urinary retention developed as early as the 16th day after injection. Animals died with tumor or were sacrificed from the 25th to the 61st day. A wide distribution of tumor deposits was found at autopsy. They occurred in the lumbar vertebral bodies from L_2 to L_5, inclusive, in the vertebral canal with direct invasion of the spinal cord, in the pituitary body, ileum, pectoral muscles, and lungs.

INFLUENCE OF SEX HORMONES ON DEVELOPMENT OF HEPATOMAS IN AXC RATS INGESTING 2-DIACETYLAMINOFLUORENE.

Harold P. Morris, H. I. Firminger, and C. Dubnik Green. (National Cancer Institute, Bethesda, Md.)

The induction of hepatomas in strain AXC rats by feeding diacetylaminofluorene (di AAF) was influenced by the sex hormones. AXC males were considerably more susceptible to the development of hepatomas than AXC females. Castrated males and castrated females were resistant, while castrated females given testosterone proprionate were nearly as susceptible as normal males. Castrated males receiving diethylstilbestrol showed resistance similar to normal females.

Lesions in the liver, studied by means of repeated liver biopsies obtained by laparotomy, developed 4–6 weeks after ingesting a diet containing 12 per cent protein and 0.05 per cent di AAF for 1 month. These lesions were largely restricted to intact males and testosterone-treated castrate females. The lesions were characterized histologically by proliferation of small bile ducts and early cirrhosis, associated with areas of hepatic cell regeneration, often without distinct lobular architecture, and sometimes with nodules which gradually disappeared upon withdrawal of the carcinogen. Continuous ingestion of di AAF resulted in early appearance of hepatomas and in early death, whereas ingestion of carcinogenic diet continuously for 1 month followed by intermittent intervals on the control and the experimental diet increased survival time and also produced hepatomas in susceptible animals.

A FLEXIBLE THREAD SOURCE OF 1.25 MILLION-VOLT GAMMA RAYS FOR THERAPY.

Joseph L. Morton,* George W. Callendine, Jr.,* and William G. Myers. (Departments of Radiology and Medicine, The Ohio University Medical Center, Columbus, Ohio.)

Cylinders of cobalt 0.05 × 0.30 cm. were exposed to neutrons in the pile until they emitted gamma-ray fluxes initially equivalent to that from either 0.21 or 0.42 mg. of radium. During each disintegration the Co-60 emits a beta particle and two gamma rays of 1.17 and 1.33 million electron-volts. The half-life is 5.3 years. Because the beta particles have a maximum energy of only 0.32 Mev, it becomes possible to use radiocobalt to

supplant radium advantageously in interstitial therapy, since the betas are absorbed by thicknesses of a sheathing filter only one-tenth as great as that required to remove all the beta particles emitted by the disintegration products of radium.

The radioactive cobalt cylinders are slid into nylon tubing and sealed in place by touching the ends of the loaded segment with a hot iron. The excess tubing at each end is warmed and stretched into strong filaments to thread onto surgical needles. The flexible radioactive threads enable the cancer-therapist to sew sources of million-electron-volt gamma rays into any radiation-sensitive tumor site accessible to a needle. A typical application has been the transfixion of the portions of iliac lymph glands not removable during a pelvic operation for malignancy, with the active segments of one or more of the radioactive threads. The filamentous extensions are brought together and held in a hemostat and the incision closed to them from both ends. After a week of exposure the radioactive sources are easily withdrawn through the incision without reopening the abdomen.

THE OXIDATIVE DEMETHYLATION OF N-METHYLAMINOAZO DYES BY RAT LIVER HOMOGENATES.

Gerald C. Mueller and J. A. Miller. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

In the intact rat the N-dimethyl dyes related to the hepatic carcinogen 4-dimethylaminoazobenzene are stepwise demethylated. In this sequence the first demethylation is reversible, and the N-dimethyl and N-monomethyl dyes are equally carcinogenic. However, the second demethylation appears to be irreversible, and the primary aminoazo derivatives have proved essentially inactive wherever tested.

Similarly, the stepwise enzymatic demethylation of these N-dimethyl dyes has been observed *in vitro* with properly fortified rat liver homogenates. In these systems the removal of the first methyl group proceeds slowly, whereas the demethylation of the monomethyl dye takes place rapidly. An investigation of the co-factor requirements of the homogenate system has revealed that triphosphopyridine nucleotide, diphosphopyridine nucleotide, and adenosine triphosphate are required for optimal demethylation of the monomethyl dye. In an atmosphere of nitrogen, the rate of demethylation was decreased to approximately one-fifth of that observed in air. In accord with an oxidative removal of the N-methyl group, it has been possible, with the aid of semicarbazide as a trapping agent, to account for the methyl groups removed by the isolation of stoichiometric amounts of formaldehyde from the reaction mixture. Thus, the intermediate in this oxidative demethylation may be the N-methylol or some closely related derivative.

The implication of these *in vitro* findings in reference to the problem of azo dye carcinogenesis will be discussed.

SPONTANEOUS CAT TUMORS.

R. M. Mulligan. (Department of Pathology, University of Colorado, School of Medicine, Denver, Colo.)

Among 66 cats with tumors, the sex was male in 28 and female in 36; the age was less than 5 years in 9, 5–10 in 24, 11–14 in 21, and 15–20 in 10. Sex and age were unknown in 2. Sixty-two cats had 1 tumor, and 4 had 2. Data for breed were inconclusive. The types of 54 of the neoplasms were as follows: squamous-cell carcinoma, 11; fibrosarcoma, 7; sweat gland adenoma and cystadenoma, 6; sweat gland carcinoma, 6; lymphosarcoma, 5; infiltrating mammary duct carcinoma, 5; mast cell sarcoma, 4; epidermal inclusion cyst, 4; basal cell carcinoma, 2; ceruminous gland adenoma, 2; and fibrous papilloma, 2. Of the remaining 16, 4 were of uncertain origin or type, and 12 were as follows: intrahepatic bile duct cystadenoma, fibroma, mucinous adenocarcinoma of the colon with metastases in a regional lymph node, malignant neurilemoma, ceruminous gland cyst, adenocarcinoma of pancreas with metastases to the liver, leiomyoma of the uterus, adamantinoma of the mandible, synovial sarcoma, apocrine gland adenoma, sebaceous gland adenoma, and mesenteric hemangiosarcoma with hepatic and splenic metastases.

The 70 tumors studied were received between 1947 and 1950 in the course of collection of spontaneous dog tumors, a project supported by grants from the National Cancer Institute.

CLINICAL EVALUATION OF A SERO-FLOCCULATION REACTION IN 300 CASES OF HEAD AND NECK MALIGNANCIES.

Claude S. Mumma,* George C. Hall,* Harry S. Penn, Andrew H. Dowdy, and A. W. Bellamy.* (Tumor Control Center, Surgical Service, Wadsworth General Hospital, Veterans Administration Center, and the Department of Radiology and Surgery, University of California, School of Medicine, Los Angeles, Calif.)

This is to report on approximately 300 cases of various malignancies involving the head and neck, using the sero-flocculation reaction as an aid in the

diagnostic procedure. An arbitrary grading of lesions was used in the breakdown for clinical evaluation. The antigen consisted of the unsaponifiable fraction derived from human cancerous tissue. About half of the sera were tested simultaneously with a fraction synthesized by one of us (H.S.P.) from bile acids. The results obtained in both tests gave above 90 per cent correct diagnoses in biopsy-proved malignancies. Normal controls gave a maximum of 1 per cent presumably false-positive reactions. Certain benign conditions, such as papillomas of the cords, leukoplakia, etc., gave essentially negative reactions. Other diseases, however, gave as high as 12 per cent false-positives. Some sera in the latter category could be ruled out by serial dilution of the sera in the test procedures.

THE EFFECTS OF ENDOCRINE CHANGES ON THE EPITHELIUM OF THE CERVIX UTERI IN RATS. H. E. Nieburgs. (Department of Clinical Cytology, Medical College of Georgia, Augusta, Ga.)

In a recent survey, 200 women with preinvasive and invasive cervical cancer presented endocrine changes of protracted estrogen activity with increased pituitary function (in press).

To reproduce this condition experimentally, a section of the cervix comprising the squamous and columnar junction was transplanted into the spleen, together with ovarian tissue. In addition, bilateral or unilateral ovariectomy was performed. Cervical implants were made by auto- and heterotransplantation from young and old rats to rats of different age. In a certain number, unilateral adrenalectomy was performed in addition. All animals were divided into three groups, receiving, respectively, thyroid, thiouracil, and stilbestrol. A fourth group was kept as control.

In another group of rats, epithelial growth changes in the cervix were studied *in situ*. The animals left intact or ovariectomized were treated with thiouracil and stilbestrol. In another group, thyroidectomy was performed in addition to ovariectomy. A part of these animals received stilbestol only, while another group was placed on thyroid and stilbestrol treatment. Controls were used for each group. Thiouracil was given to produce a state of hypothyroidism with increased release of the pituitary fraction from the basophile cells. The animals used were white male and female rats of the Wistar strain. All rats were treated for a period of 12 months. At autopsy, the spleen with the transplanted cervical and ovarian tissue, the intact cervix with uterine horns, and all glands of internal secretion were removed and studied by serial sections. The histological changes will be presented and discussed.

LIP TUMORS IN FISHES KEPT IN CAPTIVITY. Ross F. Nigrelli. (New York Aquarium, New York Zoölogical Society, New York, N.Y.)

Lip tumors in fishes have been described as papilloma, epithelioma (epidermoid carcinoma), fibroma, and fibro-sarcoma. Fishes with fleshy, movable lips are more susceptible to papilloma and epithelioma, while those with thin, fixed lips show a higher incidence of fibroma and fibro-sarcoma. In captivity, fishes have a tendency to injure their snouts against the walls of the tank. Ulcerated lesions often appear on one or both lips. In the majority of cases there is normal healing, but sometimes a tumorous growth appears at the site of ulceration. Four species of fishes studied in the present series showed tumors which were identified as follows: fibroma with chondrification in Pike Characin (*Acestrorhynchus falcatus*); simple fibroma in rainbow trout; papilloma in Climbing Perch (*Anabas scandens*), in which the epithelial elements involved were mucous cells; and papilloma in dwarf gourami (*Colisa lalia*), in which the epithelial elements and arrangement of cells were typical.

THE EFFECT OF PARTIAL HEPATECTOMY ON LIVER TUMOR FORMATION IN RESPONSE TO 4-DIMETHYLAMINOAZOBENZENE. Alex B. Novikoff, Bjarne Pearson, and Lorraine Korson.* (Departments of Pathology and Oncology and of Biochemistry, University of Vermont, College of Medicine, Burlington, Vt.)

The effect of partial hepatectomy on tumor formation in 150–225-gm. male rats (Sprague-Dawley) was studied by feeding azo dyes either prior to or following the surgical removal of the median and left lateral lobes of the liver.

When dye feeding preceded surgery, 4-dimethylaminoazobenzene or its 3′-methyl derivative was fed for periods ranging from 4 weeks to 4 months. The tumor incidence in 104 partially hepatectomized animals was not significantly different from that in controls that had not undergone surgery or in animals subjected to sham operation.

When dye feeding was begun following partial hepatectomy, only 4-dimethylaminoazobenzene was fed. A total of 132 animals was partially hepatectomized. The time of dye feeding ranged from 6 weeks to 4 months. The data from these experiments will be presented and their significance discussed.

More than 200 partially hepatectomized and control animals have developed tumors. An analysis of their histological character will be presented.

THE DEPHOSPHORYLATION OF ADENOSINE TRIPHOSPHATE AND OTHER PHOSPHATE ESTERS BY TUMORS. ALEX B. NOVIKOFF, ESTELLE PODBER,* and JEAN RYAN.* (Departments of Pathology, Oncology, and Biochemistry, University of Vermont, College of Medicine, Burlington, Vt.)

The intracellular distributions of the enzyme systems which dephosphorylate adenosine triphosphate (ATP) at pH 7.4, adenosine monophosphate at pH 7.4, and disodium phenyl phosphate at pH 9.1 were studied in liver tumors of rats fed azo dyes. In striking contrast to normal adult liver and regenerating liver, a major portion of these enzymatic activities was found in the microsomes (isolated by differential centrifugation from 0.88 M sucrose homogenates). All three transplantable rat tumors studied showed the same distributions of activities, as did the liver tumors.

In all the tumors studied calcium and magnesium ions were equally effective in stimulating the dephosphorylation of ATP. On the other hand, with homogenates of normal adult, regenerating, and fetal liver, magnesium was far more effective than calcium; this difference in the effect of the two ions on the homogenate results from the much greater stimulation by magnesium of mitochondrial dephosphorylation of ATP.

STUDIES OF VARIOUS TESTS FOR MALIGNANT NEOPLASTIC DISEASES. VII. SERUM INHIBITORS OF TRYPSIN AND CHYMOTRYPSIN. ANDREW C. PEACOCK* and JOHN J. SHEEHY* (introduced by Stuart Lippincott). (Clinical Trials Unit, Cancer Control Branch, National Cancer Institute; and the Department of Pathology, School of Medicine, University of Washington, Seattle, Wash.)

The levels of serum antiproteolytic substances, as measured against both chymotrypsin and trypsin (method of Kunitz), have been studied to determine whether such levels are of value in the diagnosis of malignant neoplasia. The control group (chymotrypsin) consisted of 152 healthy persons 18–66 years old (temperature, white and red blood cell counts, and x-ray of chest were normal in all). Sera from 136 patients with various types of malignant neoplasms (proved by microscopic examination), from 56 patients with benign neoplasms, and from 156 patients with various non-neoplastic diseases were studied. In the trypsin series, groups were composed of 76, 117, 53, and 132 persons, respectively. All sera were obtained after an overnight fast.

A level of inhibitor higher than that of the 95 percentile of the control group was arbitrarily considered positive, thereby giving 5 per cent false positives in the control group. On this basis, results with chymotrypsin gave the following percentage of correct diagnoses (trypsin percentages in parentheses): malignant neoplasms, 46.3 (49.6); benign neoplasms, 85.6 (85.0); miscellaneous non-neoplastic diseases, 64.7 (63.6). In the group of malignant neoplasms, the percentage of correct diagnoses was nearly twice as high in those subjects known to have metastases as it was in those in whom metastases were believed absent.

The lack of specificity and high incidence of false-negative results seems to indicate that use of antiproteolytic values in a mass screening test is not justified.

These and other data suggest the identity of trypsin and chymotrypsin inhibitor.

SUBMICROSCOPIC PARTICLES FROM THE CYTOPLASM OF NORMAL AND LEUKEMIC MOUSE SPLEEN. M. L. PETERMANN and M. G. HAMILTON.* (Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Extracts of cytoplasm were prepared by homogenizing the spleens in 0.88 M sucrose or in phosphate-chloride buffer and removing the tissue debris, nuclei, and mitochondria by centrifugation. Up to 90 per cent of the pentosenucleic acid (PNA) in these extracts could be sedimented at 100,000 *g*. The PNA to nitrogen ratio of the sediments increased with time of centrifugation, the highest value obtained on normal spleen being 1.2, and on leukemic spleen, 1.8. In the optical ultracentrifuge the resuspended particles were very polydisperse, but showed one sharply defined component of high sedimentation rate, whose concentration was increased in extracts of leukemic spleen.

Electrophoretic analyses on whole extracts showed at least eight components at pH 8.6. In the extract from leukemic spleen there was an increase in the components of higher mobility, as might be expected from its higher PNA concentration. Sedimentation of the nucleoproteins removed much of this material, leaving supernatants which were very similar for normal and leukemic spleen. When the resuspended sediments were analyzed electro-

phoretically, the particles from leukemic spleen showed not only a general shift to an increased concentration of the components of higher mobility but also a definite increase in the mobility of one of the major components.

THE METABOLISM OF N-(*p*-TOSYL)-2-AMINOFLUORENE-S^{35} IN THE RAT. Francis Earl Ray and Mary Frances Argus.* (Cancer Research Laboratory, University of Florida, Gainesville, Fla.)

Other investigators have established that derivatives of 2-aminofluorene such as the acetyl, diacetyl, benzoyl, carboxymethyl, and hemisuccinoyl are carcinogenic. The N-(*p*-tosyl)-, or 2-(*p*-toluenesulfonyl)-aminofluorene, however, is noncarcinogenic. The acetyl group has been shown to be removed *in vivo*, but no enzyme system is known that will remove the tosyl group. It seems probable, therefore, that 2-aminofluorene is the actual carcinogen. If this is the case, the metabolism of N-(*p*-tosyl)-2-aminofluorene should differ materially from that of 2-acetylaminofluorene.

Radioactive N-(*p*-tosyl)-2-aminofluorene-S^{35} was synthesized and fed in coconut oil to adult rats by stomach tube. Urine and feces were collected as long as radioactivity could be detected in the urine. Liver, kidney, stomach, intestines, and blood were also analyzed. Twenty-four hours after administration, about 2 per cent remained in the stomach and 38 per cent in the intestines. The liver contained about 1 per cent and the kidney and blood plasma about 0.1 per cent. At the end of 66 hours, radioactivity had completely disappeared from the blood, while a total of 0.5 per cent of radioactivity had been excreted in the urine. This compares with about 25 per cent excreted in the urine from 2-acetylaminofluorene. Over 90 per cent was found in the feces. By the isotope dilution method it was shown that the original compound was present unchanged in the feces but that in the urine it had been metabolized. No radioactive inorganic sulfate was present. Over 92 per cent of the ingested dose was accounted for.

These results are further evidence in support of the theory that hydrolysis to 2-aminofluorene is a prerequisite to carcinogenesis.

THE PRESENCE OF MICROÖRGANISMS IN TUMORS AND OTHER TISSUES OF MICE. H. Christine Reilly and Hazel M. Overby.* (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Tumors, livers, and spleens of mice bearing a variety of transplanted neoplasms and tissues from leukemic mice have been cultured on Sabouraud's agar, and the plates incubated at 30° C. for at least 1 month. Microörganisms of both bacterial and fungal types have been isolated from the normal tissues, as well as from the tumors. Although a higher percentage of tumors yielded microörganisms than did the normal tissues of the same mice, no significant difference in types of organisms present in neoplastic and normal tissues was observed. No evidence has been obtained to indicate that any one microbial type is associated with the development of a particular tumor.

Tissues of normal, nontumor-bearing mice have not shown the presence of microörganisms.

The results obtained appear to indicate that animals subjected to the stress of bearing tumors are particularly susceptible to the invasion of microörganisms.

THE INHIBITORY ACTION OF METHYLCHOLANTHRENE ON RATS FED THE AZO DYE 3′-METHYL-4-DIMETHYLAMINOAZOBENZENE. Howard L. Richardson* and Lew Cunningham (introduced by A. Clark Griffin). (University of Oregon Medical School, Portland, Oregon, and Stanford University, Calif.)

It was accidentally discovered during the course of other experiments that, when small amounts of 20-methylcholanthrene in benzene were applied vaginally, there was inhibition of liver cancer in rats fed the azo carcinogenic dye, 3′-methyl-4-dimethylaminoazobenzene (m′MeDAB).

In a controlled group of fifty-four animals fed m′MeDAB, 0.06 per cent in a basal diet, it was found that histologically proved liver cancer developed in 100 per cent of the rats of the Sprague-Dawley strain in a period from 15 to 29 weeks. These animals had lung metastasis in 74 per cent and peritoneal metastasis in 87 per cent.

The anticarcinogenic inhibitory action of 20-methylcholanthrene was demonstrated by the above investigators independently. In one group of twenty-four animals 0.06 per cent m′MeDAB was given in a basal diet. These animals received weekly vaginal administration of 0.01 ml. of 0.6 per cent 20-methylcholanthrene in chemically pure benzene. After 33 weeks of continuous carcinogenic administration four rats (17 per cent) developed primary liver cell carcinoma. In none was there evidence of bile duct carcinoma nor was metastasis histologically found in any organs.

IONIC ENHANCEMENT AND INHIBITION OF ENZYMES FROM MOUSE MELANOMAS. VERNON RILEY. (National Cancer Institute, Bethesda, Md.)

Various factors influencing the assay of cytochrome oxidase, succinoxidase, and dopa oxidase of the Harding-Passey and Cloudman S91 mouse melanomas have been quantitatively determined. Enhancement, exceeding 40 per cent, for the cytochrome system was obtained with 0.05 M NaCl, while with the succinoxidase system the same salt induced an inhibition with a rapidly increasing function from about 0.01 through 1.0 molarity. Physiological concentrations (0.9 per cent) inhibited up to about 50 per cent. Reversibility of this effect following removal of the salt was shown. These have been mostly single salt determinations imposed on aqueous cell-free tumor extracts.

The inhibition phenomena were also observed for the substrates, sodium succinate, at concentrations exceeding 0.08 M; and for dopa (DL-dihydroxyphenylalanine), at concentrations in excess of 0.008 M. Inter-relations between the enzymes and their substrates in respect to activities with varying concentrations of both have been studied and optimum conditions for maximum activity established for the three systems from mouse melanomas. The problem of proper autoxidation correction for substrate in the presence of the enzyme and tissue components has been approached by measurement of oxygen consumption of the substrate alone and combined with the enzyme extract inactivated by heat or chemical means. It was found that, while the melanoma tissue extract depressed the autoxidation of dopa, it enhanced *p*-phenylenediamine autoxidation.

INFLUENCE OF ACTH ON URINARY SECRETION OF AMINO ACIDS. EUGENE ROBERTS, ETHEL RONZONI,* and SAM FRANKEL.* (Wernse Laboratory of Cancer Research and Department of Neuropsychiatry, Washington University School of Medicine, St. Louis, Mo.)

A study was made of the amino acid excretion of physiologically normal subjects during periods when ACTH was given daily and for periods before and after the administration of the hormone. Two-dimensional paper chromatograms were made of protein-free dialysates of urine corresponding to 1×10^{-4} of the 24-hour excretion before and after acid hydrolysis. On the first day after the administration of the hormone there was a marked acceleration in the excretion of free amino acids. The increased excretion continued throughout the entire period of administration of the hormone. There was a precipitous drop in free amino acid excretion on the first day after cessation of the treatment to levels slightly below those found during the control period. During the treatment the contents of free glutamine, alanine, threonine, glycine, serine, lysine, histidine, and taurine increased markedly. There were smaller increases in amounts of glutamic and aspartic acids, glycine, and cystine. The total quantities of a number of the constituents found after acid hydrolysis also were greater. Asparagine, which was not detected during the control or postexperimental periods, appeared in large amounts during hormone treatment. Of particular interest was the finding that a much greater proportion of the total glycine excreted was found in the free form during ACTH administration than during the control periods. Microbiological determination of a number of the amino acids gave results which were concordant with the chromatographic findings.

AGE OF THE HOST AND OTHER FACTORS AFFECTING THE PRODUCTION WITH URETHAN OF PULMONARY ADENOMAS IN MICE. STANFIELD ROGERS (introduced by Peyton Rous). (The Rockefeller Institute for Medical Research, New York, N.Y.)

Young, rapidly growing mice are greatly more responsive to the adenoma-inducing influence of urethan than those just arriving at maturity. This is manifest both in the proportion of animals developing the tumors and in the number of tumors per individual. An amount of urethan per gram body weight which suffices to induce adenomas in only an occasional 8-week-old animal will cause them to appear in quantity in more than half the 3-week-old mice injected. There is an almost absolute inverse correlation between the rate of growth of the pulmonary tissue between the ages of 2 and 10 weeks and the response to urethan in terms of adenomas. Hence, the conclusion seems justified that the natural proliferative activity of the alveolar cells during youth plays a major part in the formation of the tumors. After the sixth week the age differences become relatively slight, yet there is reason to think that they continue in some degree as life goes on.

Urethan does not have the effect of promoting multiplication of the cells it has rendered neoplastic, its whole role being to initiate neoplastic change.

The abnormalities induced by urethan in the nuclei of normal and neoplastic cells have suggested to previous workers that it brings about the

adenomatous state by acting upon the nucleus. But experimentation shows that a karyolytic poison, colchicine, fails to influence the yield of adenomas to urethan, when administered concurrently. Fasting at this time does not alter it either, although it markedly reduces mitotic activity.

THE EFFECT OF CALORIC RESTRICTION AND OTHER PROCEDURES ON MITOSES IN MOUSE EPIDERMIS. H. P. Rusch, P. Gausewitz,* and R. K. Boutwell. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

To study the mechanism of caloric restriction on tumor formation, the effect of various procedures on cell division in mice maintained on high and low calorie diets was determined. The influence of the adrenal was observed because of the adrenal stimulation noted in mice kept on diets restricted in calories. The ovaries were removed in adrenalectomized mice to eliminate the compensatory effect of these organs. Since 3,4-benzpyrene (BP) has been shown to stimulate cell division in epidermis, its effect on mitosis in the skin of mice on low caloric intake was determined. After 4–6 weeks on the experiment, 3–6 mice from each group were killed, and the number of mitoses were counted in 10,000 nuclei of the epidermis from the inner surface of each ear.

The number of mitoses was 16.5 (9–26) per 10,000 nuclei in full-fed mice and 5.7 (1–12) in those on a restricted diet. When a 0.3 per cent solution of BP was applied to the skin twice weekly for 6 weeks, mitoses were increased to 43.6 (36–55) in the full-fed mice and to 18.0 (14–21) in the restricted mice. Thus, BP induced a threefold stimulation of mitosis in mice on a low caloric diet, but the number of dividing cells was still less than half that noted following the application of BP to the epidermis of mice on a full diet. The painting of an aqueous adrenal cortical extract on the skin of mice on a full diet, in amounts sufficient to inhibit the growth of hair, had no influence on mitosis in skin. The removal of the ovaries and adrenals in full-fed or restricted mice was also without effect on cell division.

THE DISTRIBUTION OF 3′-METHYL-(C^{14})-4-DIMETHYLAMINOAZOBENZENE IN THE RAT. David A. Salzberg,* Satoshi Hane,* and A. Clark Griffin. (Department of Chemistry, Stanford University, San Francisco, Calif.)

The use of 3′-methyl-4-dimethylaminoazobenzene, labeled with carbon-14 in the 3′-methyl position, for studies of the metabolism of this carcinogen in the albino rat is reported in this paper. This compound was administered orally to rats in a single large dose, or mixed in the diet (0.06 per cent), and fed for 21 days.

Of the blood fractions tested, the formed elements had the greatest amount of radioactivity. Liver samples were homogenized and fractionated into the particulate cellular components and carbon-14 activity measured. Liberation of liver–protein-bound dye was accomplished by the alkaline hydrolysis method (Miller, E. C., and Miller, J. A., *Cancer Research*, **7**:468, 1947). The amount of dye or dye metabolites liberated was calculated from radioactivity measurements. Values agreed with those of the Wisconsin group, which were obtained by colorimetric determination of the amine liberated by chemical reduction of bound dye. Additional radioactivity, however, was found in the residual alkaline solution after removal of the liberated dye. This radioactive component was not detectable by colorimetric methods used for the dye and amounted to almost 50 per cent of the carbon-14 activity found in the extractible fraction.

In agreement with the findings of Miller and Miller, none of the dye administered could be detected in the feces by colorimetric methods. However, approximately 20 per cent of the administered carbon-14 activity was found in the feces. No radioactivity was found in organs other than the liver of rats fed a single dose of radioactive dye and sacrificed within 96 hours. Carbon-14 activity was found in the adrenal glands after the feeding of the dye for 21 days.

Ribloflavin isolated from liver, urine, and feces contained a portion of the administered radioactivity. This has led to a study of growth-stimulating properties of 3′-methyl-4-dimethylaminoazobenzene on riboflavin-deficient Neurospora mutants.

METABOLIC CHARACTERISTICS OF MALIGNANT BONE DISEASE: UTILIZATION OF ORAL AND INTRAVENOUS CALCIUM. Albert Schilling and Estelle D. Gottesman.* (Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

The mineral metabolism of cancer patients is of three types: normal, osteolytic, and osteoblastic. The metabolism of patients with active osteolysis is characterized by markedly negative mineral balance, hypercalcinuria, hyperphosphaturia, and, in excessively rapid osteolysis, hypercalcemia. The metabolism of patients with actively osteoblastic lesions is typified by subnormal urinary calcium

excretion and by an avidity for calcium retention. The mineral metabolism is not specific for any type of tumor but is an expression of whether osteolytic or osteoblastic activity predominates.

The effect of androgen, estrogen, and castration therapy on the metabolism of patients with breast, prostatic, and other cancers is described, and the specificity of action is discussed.

Further characterization of mineral metabolism in cancer patients has been achieved by observing the utilization of orally administered calcium gluconate. Acute experiments with intravenously administered calcium gluconate are described, and the effect on serum and urinary calcium and phosphorus has been observed. The problem is being further investigated with tracer studies of Ca^{45}.

THE EXCRETION OF RADIOACTIVE STILBESTROL AND ITS METABOLITES BY PATIENTS WITH CANCER. Erwin F. Schoenewaldt* and Gray H. Twombly. (Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

Diethylstilbestrol labeled with C^{14} in one of its ethyl groups has been synthesized with a specific activity of 3.73 μc. per milligram. Permission to use this material in doses of 25 μc. or less per patient in cancer cases was granted by the Atomic Energy Commission when it was shown that in mice injected subcutaneously no appreciable amount of C^{14} appeared in the CO_2 of the expired air and that over 90 per cent was excreted either in the urine or feces within 48 hours. In cancer patients no radioactive carbon has been detected in the expired air. A moderate amount is found in the bile. Most of the radioactivity appears in the urine.

A NEW METHOD FOR THE COLORIMETRIC ESTIMATION OF URINARY CORTICOSTEROIDS. Arnold M. Seligman and Herbert A. Ravin.* (Yamins Laboratory for Surgical Research, Beth Israel Hospital, and Department of Surgery, Harvard Medical School, Boston, Mass.)

The steroids in urine were extracted with chloroform, and the corticosteroid component was converted to aldehydes by reduction with lithium aluminum hydride, followed by dehydration with potassium acid sulfate. The aldehydes were allowed to react with 2-hydroxy-3-naphthoic acid hydrazide (this reagent was developed for the histochemical demonstration of ketosteroids by Ashbel and Seligman, 1949). The excess hydrazide was made water-soluble and removed with the sodium salt of *o*-sulfobenzaldehyde, and the residual hydrazone in ethyl acetate solution was converted to a blue pigment by coupling with tetrazotized diorthoanisidine. The color density was measured with a photoelectric colorimeter and was converted to an equivalent number of milligrams of cortisone. Since the method is specific for steroids with an alpha-ketol or dihydroxy ketone side chain and does not require the tedious procedure of partition between benzene and water to separate the corticosteroids from the 17-ketosteroids, it is superior to other methods of assay.

EFFECT OF X-RADIATION AND OF RADIOMIMETIC SUBSTANCES ON THE SULFHYDRYL CONTENT OF PLASMA: RELATIONSHIP TO ADRENAL ACTIVITY FOLLOWING STRESS. Bernard Shacter. (Bio-Medical Branch, U.S. Naval Radiological Defense Laboratory; Laboratory of Experimental Oncology, National Cancer Institute; and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

Sulfhydryl groups have been identified with many functional activities, including cell growth. Decreased concentrations of sulfhydryl groups in human serum have been observed in various clinical conditions. The possibility was suggested that the decreases were related to concomitant changes in adrenal activity. Recently, considerable interest has been directed toward the relationship between x-radiation and sulfhydryl groups. A study was therefore undertaken on the effects of x-radiation and of radiomimetic substances on the sulfhydryl content of rat plasma. In addition, because of the possible relationship between sulfhydryl groups and adrenal activity, the effect of adrenalectomy and of sham operation on plasma sulfhydryl levels was also determined.

Exposure of rats to 750 r total body x-radiation was followed after several days by a marked decrease in the sulfhydryl content of the plasma. Similar effects were observed following administration of the radiomimetic substances, nitrogen mustard and trisethylene triazine. A decrease in plasma sulfhydryl levels was observed within 24 hours after surgical treatment of rats. Since the plasma sulfhydryl changes occurred concomitantly with the initiation of active tissue regeneration, it is suggested that decreased plasma sulfhydryl levels may be indicative of an increased demand for sulfhydryl groups in tissues during periods of accelerated cellular proliferation, and that the plasma proteins may serve as sources of sulfhydryl groups for tissue requirements.

To clarify the relationship of sulfhydryl groups to adrenal activity, a hypothesis is proposed that one of the functions of the adrenals is to assist in maintaining the sulfhydryl equilibrium in the tissues.

PRODUCTION AND TRANSFER OF MYELOGENOUS LEUKEMIA IN THE RAT. HARRY SHAY, MARGOT GRUENSTEIN,* CHARLES HARRIS,* and LILLY GLAZER.* (Fels Research Institute, Temple University School of Medicine, Philadelphia, Pa.)

In experiments in which methylcholanthrene was instilled into the stomach of random-bred Wistar strain rats, three cases of myelogenous leukemia developed. Attempts to transfer the leukemia to 40-day-old rats failed. Transfers to animals younger than 7 days were successful. Two-tenths cc. of blood, or of liver or spleen cells, was injected intraperitoneally for transfer. Diagnosis of induced or transferred leukemia was always made first from peripheral blood and subsequently corroborated by bone marrow smears and tissue sections.

The average latent period was 6–10 weeks, with occasional "takes" at 5 weeks and sometimes after as long as 5 months.

White blood counts ranged from 50,000 to 292,000, with 7 per cent to 30 per cent of myelocytes.

The leukemia is chronic in type and on transfer consistently resulted in a chloroleukemia with a histologic picture similar to that described by Wilens and Sproul (*Am. J. Path.*, **12**:249, 1936) and by Oberling, Guerin, and Guerin (*Bull. Assoc. franç. l'étude cancer*, **28**:214, 1939).

To date, transfer by means of blood has been successful in our Wistar strain in 26 of 31 animals (84 per cent), with liver tissue in 16 of 30 (53 per cent), with spleen in 7 of 18 (39 per cent). It also has been transferred by means of blood to 8 of 13 (62 per cent) random-bred Sherman strain rats and by liver tissue to 7 of 17 (41 per cent) random-bred Long-Evans strain rats.

THE EFFECT OF COBALT ON THE ANEMIA OF CANCER. SHU CHU SHEN* and FREDDY HOMBURGER. (Cancer Research and Cancer Control Unit, Departments of Surgery and Medicine, Tufts College Medical School, Boston, Mass.)

In order to explain the erythropoietic effect of cobalt, observed in some of the anemic cancer patients who received cobaltous chloride orally, the effect of this hematopoietic agent on red blood cells *in vitro* was investigated. Normal human erythrocytes in serum were incubated for 24 hours at 37° C. with 0.2 per cent cobaltous chloride. The formation of methemoglobin and the increase in osmotic and mechanical fragility of the erythrocytes were noted at the end of incubation.

Further study of the cause of methemoglobin production in the red blood cells by cobalt indicated that this is due to the impairment of the reducing activity of the cells rather than to the formation of oxidants. This is shown by the facts (*a*) that the required amount of methylene blue, in the presence of glucose, is unable to accomplish the complete reconversion to hemoglobin of the methemoglobin formed in the cells by cobalt chloride, in contrast to the complete methylene blue reconversion of methemoglobin, formed by *p*-aminophenol which is a known oxidant, and (*b*) that methemoglobin formation, when the blood is incubated with cobalt or *p*-aminophenol, is inhibited in the presence of a suitable amount of ascorbic acid.

It is postulated that the erythropoietic effect of cobalt choride results indirectly through its interference with the cellular reducing enzyme systems, which may cause a relative anoxia of the bone marrow.

DEVELOPMENT OF TUMOR-LIKE STRUCTURE AFTER IMPLANTATION OF PARTS OF AXOLOTL EMBRYO INTO THE EYE OF ADULT FORMS. E. A. SHEREMETIEVA-BRUNST,* V. V. BRUNST, and FRANK H. J. FIGGE. (Department of Anatomy, University of Maryland, School of Medicine, Baltimore, Md.)

Parts of embryos 2 days before and 5 days after hatching were implanted into nine eyes of five adult axolotls (*Siredon mexicanum*). In all animals an active growth of these grafts was observed during the first few weeks after the implantation. Later, three grafts became reduced in size and disappeared completely. Others became relatively large (the largest was $9 \times 7 \times 5$ mm.). All grafts had the appearance of tumors. Some of these "tumors" were transplanted into the eyes of other adult animals. Most of the transplants took, but later the majority of them did not show any growth. The rate of size increase of the transplants was poor compared to that of the implants. Later all the growing transplants showed a tendency to reduce and disappear.

Histological examination of pieces of actively growing grafts showed that in some cases tissue consisted of a mixture of heterogenous cells with cartilagenous inclusions of irregular form. In other cases, the graft consisted of only homogenous con-

nective tissue. In all cases, grafts had many blood vessels and were covered with epithelium, but an organization similar to the embryo structure was not observed. They resembled tumor-like structures. Examination of only one eye revealed some organization in the proximal parts of the graft (neural tube and cartilage), but in the distal portion of the same graft a true tumor-like structure was observed.

These preliminary experiments show that under certain conditions normal embryonic tissues lose their organization and acquire tumor-like structure.

BLOOD HISTAMINE IN MYELOGENOUS LEUKEMIA. MICHAEL B. SHIMKIN, HOWARD R. BIERMAN, B. V. A. LOW-BEER,* PRISCILLA M. WHEELER,* and FRANZ R. GOETZL.* (Laboratory of Experimental Oncology, National Cancer Institute, and the Division of Medicine, University of California School of Medicine, San Francisco; Division of Radiology, University of California School of Medicine, San Francisco; and Department of Medical Research, Permanente Foundation, Oakland, Calif.)

Observations were made on the blood histamine in 30 patients with myelogenous leukemia. In comparison with values of 2–10 μg/100 cc found in normal individuals, blood histamine in myelogenous leukemia may be increased to over 2,000 μg/100 cc.

Since most of the blood histamine is contained in granulocytes, the data were analyzed by use of the ratio: histamine in μg/100 cc of blood divided by the number of granulocytes in thousands per cubic millimeter. This ratio exceeded 1.0 in twenty patients who lived for an average of 12 months following the initial blood histamine determination. The ratio was below 1.0 in nine cases, and the average survival period was 1.3 months. The difference was statistically significant, and it was concluded that a lowered blood histamine, as manifested by a histamine/granulocyte ratio of under 1.0, is associated with short survival.

In five patients with chronic myelogenous leukemia who lived for an average of 18 months, intravenous injection of epinephrine was followed by an increase in blood histamine. In four cases of acute manifestations of the disease with a short survival, this procedure was followed by a decrease in blood histamine.

STUDIES CONCERNING THE THROMBOPLASTIC PROPERTIES OF ROUS SARCOMA EXTRACTS. EDWARD W. SHRIGLEY, WILLIAM R. CLARK,* and CECIL B. DONNELLY.* (Department of Microbiology, Indiana University Medical Center, Indianapolis, Ind.)

Saline extracts and some Berkefeld filtrates of the Rous sarcoma, when inoculated intravenously into chicks, cause prompt death of the bird. The above observation is well known. At autopsy we have found a dilatation of the right heart, with a massive clot in the right auricle. Further examination reveals a clot which extends the length of the aorta. That this reaction is associated with the thromboplastic content of tumor extracts is suggested by the fact that such extracts will clot chicken plasma and also that heparin will completely protect inoculated chicks. Physiological saline extracts of sarcoma (1/20 by weight) possess clotting times of 18–25 seconds. Increase in these values, which may be achieved by dilution of the extract, is associated with gradual loss of "toxicity." Heparin will likewise lengthen clotting time.

Through work with celite eluants, it has been possible to obtain extracts containing 10–20 μg. of total nitrogen per milliliter, which possess thromboplastic potencies similar to those obtained from crude tumor suspensions. These eluants contain also Rous virus in proportions comparable to those found in less purified sarcoma extracts. Heparin renders these eluants "non-toxic" and does not appear to decrease the potency of the virus.

The thromboplastic properties of the Rous sarcoma are inhibited by globulin soy bean trypsin inhibitor and also by proteose navy bean trypsin inhibitor.

Studies of normal chick breast muscle have yielded extracts relatively low in thromboplastin.

Certain implications of the above findings as related to the pathogenesis of the Rous sarcoma will be discussed.

AGE AND SUSCEPTIBILITY OF THE MAMMARY GLAND OF MICE TO ESTROGEN. MARTIN SILBERBERG and RUTH SILBERBERG. (Snodgras Laboratory, Hospital Division, St. Louis, Mo.)

Sixty-three mice of strain C3H (29 noncastrates and 34 orchidectomized at 3 weeks) received 0.03 mg. α-estradiol benzoate once weekly for 5 months, from 7 months of age on (group C). The mammary cancer incidence was compared to that obtained in corresponding mice injected from the ages of 1 month (group A), or 4 months on (group B) (*Proc. Soc. Exper. Biol. & Med.*, **69**:438, 1948; *Arch. Path.*, **47**:340, 1949).

In noncastrate animals the growth stimulation

was less marked than in castrates of the corresponding age groups. In group C, 13.8 per cent of the castrates developed cancer at a mean age of 13 months. In group A the incidence was 44.4 per cent and the mean cancer age 9.7 months, and in group B the incidence was 30.8 per cent and the mean cancer age 12.8 months. In group C, 2.9 per cent of noncastrate animals developed mammary cancer at 12 months of age, as compared to 15.8 per cent at 10.7 months in group A, and 4 per cent at 17 months in group B. Microscopic studies of the mammary glands of mice that had failed to develop mammary cancer showed that the older the animal was at the beginning of treatment, the less stimulated were ductal and alveolar growth and secretions.

An inadequate latent period or inadequate dosage of the hormone could be ruled out as factors determining the results. The latter are considered to be due to a decrease with advancing age in the susceptibility of the mammary tissue to estrogenic hormone.

HISTOGENESIS OF SQUAMOUS CARCINOMAS OF THE HEAD PRODUCED BY 2-ACETYLAMINOFLUORENE WITH REFENCE TO PRE-EXISTING LESION. Stanley C. Skoryna,* Roderick C. Ross,* L. A. Rudis,* and D. R. Webster.* (Departments of Experimental Surgery and Pathology, McGill University, Montreal and National Cancer Institute of Canada.)

Because of the high frequency of head tumors in a colony of hooded rats, studies were undertaken to determine their site of origin and histogenesis.

Out of 126 rats of both sexes which survived the 6-month period of oral administration of 2-acetylaminofluorene, tumors adjacent to the external auditory canal were observed in 71 animals; in 12 of them bilaterally. The majority of them appeared between the ninth and twelfth month of the experiment, some as late as the sixteenth month.

Histologically, the tumors were squamous-cell carcinomas with marked keratinization, and frequently showed sebaceous areas. In a few instances pulmonary metastases were observed.

It was found that they arose in the auditory sebaceous organ, which is a compound sebaceous gland with secretory ducts draining into a single ostium in the external auditory canal, 2–3 mm. from the tympanitic membrane.

In older rats on a normal diet, dilatation and stasis of inspissated secretion was frequently observed in one or more lobules of this gland. The appearance was highly suggestive of obstruction of the excretory ducts. The co-existence of otitis media in all cases of tumors examined suggests that this may play a role. In a few cases bulging of tympanitic membrane of such degree as to occlude the main secretory duct was observed. It is likely, therefore, that this condition contributed to stasis of sebaceous material in the gland.

It was in lobules showing such cystic degeneration that the tumors arose in animals fed 2-acetylaminofluorene.

BLOOD PRESSURE EFFECT OF ADMINISTRATION OF REMOTE CARCINOGENS MEASURED BY FOOT PLETHYSMOGRAPHY. Stanley C. Skoryna,* L. A. Rudis,* and D. R. Webster.* (Department of Experimental Surgery, McGill University, Montreal, and National Cancer Institute of Canada.)

In recent years, a number of substances with potential general carcinogenic action has become known, including acetylaminofluorene, aminostilbene, and benzidine compounds. The variety and site of tumors produced apparently depend on the presence of some preceding lesion and the specific growth-stimulating effect of the compound on the structure in question. In addition to this localized remote action, the carcinogen appears to have a general effect on the animal.

A study has been instigated to determine the possible role of irregularities of autonomic functions during the period preceding development of malignant tumors. In this experiment, blood pressure measurements have been performed during the 6-months' period of administration of 2-acetylaminofluorene. The compound has been added to the diet of 84 hooded rats, 7 weeks old. The type of tumors produced and incidence, varying from 80–90 per cent, have been determined previously on animals from the same colony. Systolic and venous pressures have been measured at weekly intervals with photoelectric tensometer by means of foot plethysmography.

It was found that 2-acetylaminofluorene has a progressively hypotensive effect during the induction period of tumors, more pronounced in males than in females. Measurements on control animals on normal diet showed increase in blood pressure in the same period of time.

The hypotensive effect of the carcinogen is correlated with clinical observations as to the frequency of hypotension preceding human neoplastic diseases.

PATHOGENESIS OF FELINE LUNG TUMORS PRODUCED BY 2-ACETYLAMINOFLUORENE. Stanley C. Skoryna,*

L. A. RUDIS,* and D. R. WEBSTER.* (Department of Experimental Surgery, McGill University, Montreal and National Cancer Institute of Canada.)

The carcinogenic effect of 2-acetylaminofluorene on cats has been investigated. The drug has been administered to a group of cats by daily addition to diet. The initial dosages have been 40 mg/day for the first 6 months and 50 mg/day for the following period. In the sixteenth to eighteenth month of administration, multiple pulmonary tumors have been observed in four cats. The only changes observed in other organs was moderate liver cirrhosis noted in the first months of administration of the carcinogen.

Histologically, the tumors appeared to differ from those produced by 2-acetylaminofluorene in other species. Multiple small tumor nodules were scattered through the lungs. The cells were of poorly differentiated character, mostly appearing like those of a polymorphous sarcoma. Tumor thrombi in the vessels were frequent. As the cats' lungs are frequently the site of chronic inflammatory lesions, it was impossible to determine whether these were preexisting or secondary to the tumor formation.

Spontaneous tumors of the cat will be briefly discussed.

THE COAGULATION FACTORS IN PLASMA OF CANCER PATIENTS.

WILLIAM BROWN SMITH,* LOUIS ROSENFELD,* and GEORGE Y. SHINOWARA* (introduced by E. von Haam). (Department of Pathology, Ohio State University, Columbus, Ohio.)

Fibrinogen levels, prothrombin levels, and the thromboplastic plasma component were determined in the blood plasma of treated and untreated cancer patients. It was found that the fibrinogen was elevated up to 3 times the normal in untreated cases and often increased further during treatment with x-ray and radioactive cobalt. Prothrombin levels and the thromboplastic plasma component remained within normal limits.

RESPONSES OF MICE TO CARCINOGENIC DERIVATIVES OF PETROLEUM.

WILLIAM E. SMITH and DOUGLAS A. SUNDERLAND.* (New York University and The Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Over a very considerable range (1–30 paintings), the yield of tumors was not proportional to the number of paintings of a carcinogenic oil. Groups of mice painted 1, 3, or 12 times yielded essentially as many tumor-bearing animals as did groups painted 20 or 30 times. The animals were not inbred, and for this reason may have presented opportunities to observe differences in the skin tumor-forming abilities of individuals. Detection of these differences by repeated painting of the same material became obscured, however, at a point exceeding 30 applications. Thus, a much higher percentage of tumor-bearing animals was obtained by 80 applications than by 30.

Individual differences in the formation of skin tumors were also observed by the painting of different types of oils. Although the time of appearance of tumors was generally shorter in proportion to the carcinogenic potency of the test material, occasionally mice yielded tumors in response to a weakly carcinogenic oil as quickly as with highly potent oils. Furthermore, oils that elicited tumors quickly in a high percentage of mice often failed to cause tumors in certain cases despite long-continued painting. Hence, some individuals are unusually responsive, while others are unusually resistant to the unknown carcinogenic material in these petroleum derivatives. The causes of these differences remain to be explored.

The histology of the cancers was not related to potency of the inducing material, but there was an inverse relation between potency and the time elapsing between the first appearance of papilloma and the secondary malignant change.

FIVE ALLELES AT THE HISTOCOMPATIBILITY-2 LOCUS IN THE MOUSE AS DETERMINED BY TUMOR TRANSPLANTATION.

GEORGE D. SNELL* (introduced by Nathan Kaliss). (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)

Previous studies have proved the existence of a genetic locus on chromosome number 9 of the mouse, closely linked with the genes for fused tail (*Fu*), kinky tail (*Ki*), and brachyury (*T*), which is of major importance in determining susceptibility and resistance to tumor transplants. This study is concerned with the identification of alleles at this locus.

Crosses were made of the type (M × F) × N where M and N are any two inbred strains, and F is a stock carrying *Fu* (or *Ki* or *T*). The offspring were inoculated with a tumor from stock M. By simple genetic reasoning it can be shown that where, in this cross, *Fu* and resistance show association, M and N must carry *different H-2* alleles. On the other hand, where there is no association, M and N probably carry the *same H-2* allele. By this method, new alleles can be identified and their distribution in standard inbred strains mapped.

Five alleles have been identified. These, together with the strain in which they occur, are: *H-2* (A strain), *H-2*^{d} (dba strain, subline 2, and B alb C strain), *h-2*^{b} (C57BL strain), *h-2*^{p} (P strain), and *h-2*^{k} (a kinky tail strain and probably also the CBA strain). The *h-2*^{k} allele is of particular interest in that the heterozygote *H-2*^{d}/*h-2*^{k} will grow A strain tumor 15091a and hence is equivalent to *H-2*. The allele *H-2* is thus apparently composed of two components.

All alleles so far detected behave as dominants in crosses; there is no complete recessive.

METHODS OF PREPARATION AND ANTIGENIC ACTIVITY OF ESTROGEN-PROTEIN CONJUGATES. Douglas H. Sprunt, Anna Dean Dulaney,* and Robert Conger.* (Division of Pathology and Bacteriology, University of Tennessee College of Medicine, Memphis, Tenn.)

Since abnormal steroids have been demonstrated in the urine of certain patients with cancer, it was postulated that these substances might be detected through the use of specific antisera. The diagnostic value in cancer might then be established. Alpha-estradiol has been employed as the steroid for pilot tests. Crystalline alpha-estradiol was chemically coupled with the albumin fraction from sera of several species. The steroid-protein conjugates were then used for immunization of rabbits by the intravenous route. Dosages were adjusted to contain standard values of protein. The sera of these animals were used in complement fixation and precipitin tests employing the conjugates, proteins and steroids as antigens. Preliminary tests indicate that the conjugate antisera react in specific fashion with the conjugate antigens. The titers for the conjugate antigen were substantially higher than those demonstrated for the protein substance.

A COMPARATIVE STUDY OF SEVERAL TESTS WHICH MAY BE USEFUL AS HEALTH CLEARANCE TESTS. Douglas H. Sprunt, J. Warren Kyle,* and Sara Grace Richmond.* (Division of Pathology and Bacteriology, University of Tennessee College of Medicine, Memphis, Tenn.)

We have studied in over a thousand people the effect of cancer, tuberculosis, other infections, and a variety of other conditions on the following tests: the iodoacetate index, the least concentration coagulable protein, the sedimentation rate, and the hematocrit. The above diseases have been subdivided into a number of smaller categories. For example, the cancer ones have been separated into those which involved only a small local area, those which were extensive but still localized, and those which had metastasized. The tuberculosis cases were divided into groups which were primarily necrotizing and those which were predominantly proliferative. Other subdivisions were studied. Space does not permit going into the various findings in this abstract. Suffice it to say that none of these tests are specific for any particular disease. All of them give some measure of the body's reaction to injury.

We have also studied the reproducibility of these tests in conditions of both health and disease. The results of this show considerable variation in the sensitivity of these tests to minor variations in the health of the individual. This was particularly true of the sedimentation rate.

We have also studied the effect of the economic level which parallels roughly the protein level. We have as yet no conclusions in regard to this but are continuing our studies.

Our conclusion is that a possible combination of some of these tests with others may serve as a health clearance test, but further work must be done before this end can be accomplished.

THE EFFECT OF AUREOMYCIN ON TRANSPLANTED TUMORS. Boris Sokoloff and Walter H. Eddy.* (Southern Bio-Research Laboratory, Florida Southern College, Lakeland, Fla.)

E. Stokstad and T. Jukes reported that unpurified aureomycin exerts a growth-promoting activity when added in small amounts to the rations of hogs, chickens, and turkeys. The present investigation concerns the effect which purified aureomycin produces on transplanted tumors. Two types of tumors were investigated: August rat carcinoma 175-G, British brown breed of rats, and rat carcinoma R-13, black-white breed of rats. Altogether 190 rats were used for these experiments. Their average weight was 156 and 167 gm., respectively. When rats bearing 3-day-old transplants were given 1.6 mg/day/100 gm wt, for 7 days, there was a definite increase in the size of tumor in the treated animals. At the end of 3 weeks of treatment, the average size of tumor (20 rats) was 7.3 cc., while the average control tumor was 3.9 cc. In the next series of experiments, rats bearing 7-day transplants were given the same dose for 10 days with similar results (9.6 cc. for the treated animals and 6.5 cc. for the controls [40 rats]). When, however, a much larger dose was administered, the reverse effect was obtained. The rats

bearing 3-day transplants were given 8 mg/day/100 gm wt for 15 consecutive days. Four weeks after transplantation, the average control tumor was 8.3 cc., while the treated animals had an average size tumor of 4.3 cc. When a large dose of aureomycin, 8.0 mg/day/100 gm wt, was given to rats bearing the 1-day transplants for 18 days, a number of complete regressions of the growth were observed, and the average size of the tumors was even smaller: 3.7 cc. 4 weeks after transplantation. There was an average loss of weight of 22 gm. in the group of rats receiving heavy doses of aureomycin.

THE EFFECT OF FLAVONOIDS ON TRANSPLANTED TUMORS. Boris Sokoloff, W. H. Eddy,* J. Williams,* and L. Sciortino.* (Southern Bio-Research Laboratory, Florida Southern College, Lakeland, Fla.)

The disclosure of two flavonoid compounds present in the adrenal gland (Sokoloff and Eddy) suggested an investigation on the effect of flavonoids on malignant growth. August rat carcinoma, 175-G, served for these studies. Altogether, 130 rats were tested, with an average weight of 154 gm. In the first series of experiments, a flavonoid compound ($C_{36}H_{30}O_{11}$), water-soluble, was injected subcutaneously, 10 mg/100 gm for 19 days, 5 days prior to transplantation and for 2 weeks after. At the end of 3 weeks after transplantation the average size of tumor (for 20 rats) was 3.4 cc., compared to 6.4 cc. for controls. This inhibition was of a temporary nature. In another series, the same flavonoid was given to rats (20) the day of transplantation and the treatment continued for 3 weeks 10, mg/100 gm. By 4 weeks after transplantation, the average tumor was 6.1 cc., compared to 8.9 cc. for the controls. Twelve rats, all females, of an average weight of 151 gm., received subcutaneous grafts of two extra adrenal glands. The following day the tumor was transplanted. There was no significant difference in the growths. At the end of 4 weeks, the average size of tumor was 8.7 cc., compared to 9.1 cc. for the controls (10 rats). In the next series, 12 rats with grafts of two extra adrenal glands were given injections of the flavonoid compound, 10 mg/100 gm from the day of transplantation for 15 days. There was a marked inhibition, of a temporary nature. At the end of 4 weeks, the average size of tumor was 2.5 cc., compared to 8.6 cc. for controls. Discussion on the possible relationship of flavonoids and the adrenal gland is presented.

ELECTROPHORETIC STUDIES ON THE SOLUBLE PROTEINS FROM LIVERS OF RATS FED AMINOAZO DYES. Sam Sorof,* Philip P. Cohen,* E. C. Miller, and J. A. Miller. (Laboratory of Physiological Chemistry, and the McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison, Wis.)

The studies of Miller and Miller on the occurrence of protein-bound dye in the livers of rats fed the hepatic carcinogen 4-dimethylaminoazobenzene (DAB) or certain of its derivatives have pointed to the possible causal role of these proteins in aminoazo dye carcinogenesis. The protein-bound dye is found in the non-neoplastic portions of the liver throughout the stages of tumor formation as long as the dye is fed. However, the protein-bound dye cannot be detected in the dye-induced tumors, and this can be interpreted as indicating a *qualitative* difference between the proteins of the liver and liver tumor.

Over one-half of the bound dye in these livers is combined with the soluble proteins, and the present investigation was undertaken to ascertain the electrophoretic group(s) of the soluble rat liver proteins which become bound to dye following the ingestion of the potent carcinogen, 3′-methyl-DAB, or one of the very weak carcinogens, 4′-methyl-DAB and 2-methyl-DAB. Chemical analysis of the electrophoretic fractions showed that the major portion of the soluble protein-bound derivatives of these aminoazo dyes migrates with the mobility range of the h components (probably h_1). Since the h components only account for approximately 15 per cent of the total soluble liver proteins, these facts strongly suggest that metabolic derivatives of these dyes bind specific protein(s). It is of great interest that concurrent studies (Sorof and Cohen) indicate that the h components are present in greatly reduced quantities in DAB-induced tumors and other rat tumors.

THE INFLUENCE OF ADRENALECTOMY ON THE GROWTH OF TRANSPLANTED RAT LYMPHOSARCOMA. J. Stasney, K. E. Paschkis, and A. Cantarow. (Jefferson Medical College, Philadelphia, Pa.)

Adrenalectomy has been reported to enhance the development of leukemia in rats inoculated intraperitoneally with suspensions of Murphy rat lymphosarcoma cells. This report is concerned with the influence of adrenalectomy on the development of local tumors following subcutaneous injection of suspensions of these cells. Seven days after adrenalectomy, rats (maintained on 1 per cent NaCl solution) received subcutaneous inoculations in the lateral abdominal wall with a stand-

ardized suspension of lymphosarcoma cells in 0.5 ml. physiological salt solution. An equal number of intact animals, treated similarly, served as controls. All animals were sacrified 10 days after inoculation, and the tumors were dissected out, measured, and weighed. The tumors in the adrenalectomized animals were somewhat larger than in the controls, but the difference was not striking. The enhancing influence of adrenalectomy on the development of local tumor is apparently not as great as on the development of leukemia in rats inoculated with cells of the Murphy lymphosarcoma.

SELECTIVE LOCALIZATION OF SULFAPYRAZINE IN CANCER TISSUE UPON ELEVATION OF THE BLOOD SUGAR. CHARLES D. STEVENS, ANNA MARY KOCK,* MARY ANN WAGNER,* and PATRICIA M. QUINLIN.* (Department of Biological Chemistry, College of Medicine, University of Cincinnati, Cincinnati, Ohio.)

A method of precipitating in cancer tissue a compound administered at sites distant from the cancer was suggested by bringing together two facts: (*a*) many compounds are less soluble in acid than in alkali; (*b*) cancer tissue is possibly unique in becoming quite acid, often below pH 6.4, as measured by glass electrode, after glucose administration. The compound used was sulfapyrazine, which is less soluble at pH 6.4 (tumor pH) than at pH 7.4 (serum pH). Rats with Walker tumor 256 in the interscapular region were used. Aqueous sodium sulfapyrazine was injected in the hind leg, glucose intraperitoneally.

After the rats were killed, 1, 2, or 3 days after sulfa injection, sulfapyrazine was frequently grossly visible, precipitated in the necrotic tumor tissue. Sulfa concentrations in whole tumors often exceeded those in serum from blood drawn at sacrifice, occasionally by two- to threefold. In nonnecrotic portions of tumors, sulfa concentrations seldom exceeded those in serum. Precipitation of sulfa in the stomach, in the kidney tubules, and at the injection site was sometimes observed. Hide and blood contained sulfa concentrations close to those in serum. Sulfa concentrations lower than but proportional to those in serum were found elsewhere: liver, spleen, lungs, thymus, lymph nodes, thyroid, adrenals, heart, leg muscle, brain, and testes. The doses of sulfapyrazine were generally lethal. Similar but less marked localization was obtained with sulfaquinoxaline and with radioactive 2-sulfanilamido-5-iodopyrimidine. Single glucose injections do not long maintain elevated blood sugar; some success has attended injection of alloxan, dextrin, or starch.

SUSCEPTIBILITY TO FIBROSARCOMAS IN 2NHO MICE. LEONELL C. STRONG. (Yale University School of Medicine, New Haven, Conn.)

The 2NHO strain was produced by crossing mice of one of the NHO substrains (genetic constitution ppbbaaSeSe) to mice of the JK strain (genetic constitution ppbbaasese). This strain was continued for twenty inbred generations (F_1–F_{20}). At 60 days of age all mice were injected with 1 mg. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. Continuous selection against the appearance of induced fibrosarcomas was followed by keeping the descendants of the most resistant pair of mice in each generation. The average latent period for fibrosarcomas was progressively increased in the early generations, reaching the greatest value in mice of the F_4 generation. F_1 produced 50 per cent by 162 ± 7.9 days; F_2 50 per cent by 325 ± 26.4 days; F_3 50 per cent by 412 ± 18.5 days; and F_4 50 per cent by 418 ± 13.9 days. Beginning with mice of the F_5 generation and continuing to the F_{20} generation, when the experiment was terminated, there was a progressive decrease in the latent period for fibrosarcomas. Thus, biologic variation took place counter to direction of genetic selection. A total of 3,247 mice was used; 1,529 females and 1,718 males (47.1 per cent ♀♀ : 52.9 per cent ♂♂). There was no sex difference in susceptibility to fibrosarcomas. Mice of the F_{15} and F_{16} generations showed a delayed incidence of fibrosarcomas quite out of range of progressive susceptibility. 2NHO controls developed only a few lung adenomas at advanced ages (9.89 per cent at 776 days of age). Methylcholanthrene-injected mice developed 21.6 per cent lung adenomas in F_1–F_6 generations (285 lung adenomas in 1,319 mice).

EFFECT OF VARIOUS COMPOUNDS ON EHRLICH ASCITES TUMOR IN MICE. KANEMATSU SUGIURA and C. CHESTER STOCK. (Division of Experimental Chemotherapy, The Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Previous investigations with a spectrum of tumors (thirteen mouse and four rat tumors—carcinomas, sarcomas, osteogenic sarcomas, lymphosarcomas, melanoma, hepatoma) have demonstrated that aminopterin, A-methopterin, HN 2, 3-bis (β-chloroethyl) amino-methyl-4-methoxy-methyl-5-hydroxy-6-methyl pyridine dihydro-

chloride (SK 1424), triethylene melamine (TEM), 2,6-diaminopurine, 8-azaguanine, and cortisone had a definite inhibitory or destructive action on certain types of tumors. In the present series of experiments the effect of these compounds on the growth of Ehrlich mouse carcinoma, both solid form and ascitic form, was studied.

The original solid tumors were obtained by subcutaneous injection of 0.1 cc. of fresh ascitic fluid containing about one million cancer cells, and the ascites tumors by intraperitontal injection into mice. Mice regularly developed large ascites (10–20 cc.) in 7–14 days and died in 3–4 weeks. In general, the first intraperitoneal or subcutaneous injection of compounds was given 1–7 days after inoculation of tumor fragments or injection of ascites, and injection was continued for 7 days.

Daily subcutaneous or intraperitoneal injections of 0.25 mg/kg of aminopterin and 1.5 mg/kg of A-methopterin had no inhibitory effect on Ehrlich ascites tumor, both solid and fluid forms. The daily subcutaneous or intraperitoneal injections of 1 mg/kg of HN2, 5 mg/kg of SK 1424, and 0.25 mg/kg of TEM had a slight inhibitory effect on solid tumor but marked inhibitory effect on ascites. Repeated subcutaneous or intraperitoneal injections of 75 mg/kg of 8-azaguanine had no inhibitory effect on Ehrlich ascites tumor, both solid form and ascitic form. Subcutaneous injection of cortisone, 37.5 mg/kg, had a slight inhibitory effect on solid tumors and a marked inhibitory effect on ascites.

LIVER TUMOR INDUCTION IN ADRENALECTOMIZED AND DOCA-TREATED RATS FED WITH *p*-DIMETHYLAMINOAZOBENZENE.

ALEXANDER SYMEONIDIS, AMBADAS S. MULAY,* and FREDERICK H. BURGOYNE.*† (National Cancer Institute, Bethesda, Md.)

Four series of male Osborn-Mendel rats 4–7 weeks old were kept 190 days on a semisynthetic diet mixed with *p*-dimethylaminoazobenzene. These animals received various types of treatments, as follows:

	No. started on experiment	Number surviving	Experimental procedure
Series I	112	30	Adrenalectomy, saline in drinking water
Series II	41	31	Adrenalectomy, subcutaneous DOCA pellets
Series III	26	26	Intact, subcutaneous DOCA pellets
Series IV	37	37	Intact

† Present address: Misericordia Hospital, Winnipeg, Manitoba, Canada.

An additional control Series V of 27 rats was kept on a regular stock diet.

The average hormone absorption in Series II and III was 220 mg.

The animals were killed and the organs examined with the dissecting microscope. At least ten sections from each liver were examined histologically. Microscopic lesions were classified as: (*a*) atypical bile duct proliferation (ABD), (*b*) atypical liver cord regeneration (ALC), and (*c*) hepatoma (H). The liver weight in percentage of body weight was also calculated. Chemical determinations of glycogen and alkaline phosphatase were done.

	Liver lesions				Liver weights in per cent body weights	Glycogen	Alkaline phosphatase
Series	ABD	ALC	H	Total			
I	8	2	2	12	4.85	30.37	70.63
II	0	1	2	3	5.22	32.75	49.11
III	0	0	0	0	5.50	40.28	47.36
IV	8	5	7	20	4.87	37.13	58.07
V	0	0	0	0	3.84	30.61	35.06

Nodules of regenerating adrenal cortex were found in most of the animals of Series I and II bearing tumors. Distinct diminution in size of the adrenal glands due to atrophy of the cortical cells was found in Series III rats.

Notable observations were the failure of tumor development, the high liver weight in per cent of body weight, the high glycogen and the low alkaline phosphatase of the liver in the Series III animals, and the high alkaline phosphatase content of the liver in Series I and IV rats.

SUCCESSFUL GROWTH AND SERIAL TRANSPLANTATION OF HUMAN TUMORS IN RATS AND MICE IRRADIATED BY X-RAYS.

HELENE WALLACE TOOLAN. (Sloan-Kettering Institute, New York, N.Y.)

Cell suspensions prepared from 35 different human invasive neoplasms have been implanted subcutaneously in rats and mice previously subjected to radiation with X-rays by a special technic. Twenty-seven gave evidence of active growth histologically like that of the implanted material by the eighth day after implantation. Two have been transplanted for as many as nine generations, and all but three for at least three generations.

The neoplasms transplanted include a leiomyosarcoma, a synovioma, several melanomas, an ovarian carcinoma, and a carcinoma of the colon, as well as the more common epidermoid carcinomas. The last have been to date the most prolific. Five carcinomas of mammary origin failed to grow

even though female experimental animals were employed.

METHOD OF TRANSPLANTING GASTRIC MUCOSA TO THE ANTERIOR ABDOMINAL WALL OF THE RAT FOR LOCAL APPLICATION OF CARCINOGENS. EDNA W. TOOVEY,* LEON HELLER,* and D. R. WEBSTER.* (Department of Experimental Surgery, McGill University, Montreal, with Grant-in-Aid from National Cancer Institute of Canada.)

In an attempt to devise a "worthy experimental tool," it was thought that, by isolating a portion of gastric mucosa in the rat in such a way that carcinogenic substances could be applied locally, visible and consistent results might be obtained. Therefore, during the winter of 1949–50, pouches were made in the rat from the greater curvature of the glandular stomach. The pouch opened onto the anterior abdominal wall, thereby permitting carcinogenic agents to be applied directly to the gastric mucosa. These pouches, however, everted. So a technic was devised in which a "flap" or patch of gastric mucosa was placed and grew on the anterior abdominal wall. Twenty animals were operated on, and in seventeen the gastric flaps grew well on the anterior abdominal wall. When it was seen that this could be done successfully, further operations were done, and 120 animals with good patches of gastric mucosa were obtained from 182 operations completed by October 13, 1950.

The usual solvents for carcinogens, such as acetone and benzene, were tried but could not be used, as the local irritation was so great that the animals immediately licked off the irritating solvent. A search of the literature disclosed a relatively nontoxic and bland solvent, polyethylene glycol 300, which did not annoy the animals when it was applied to the gastric flap with a No. 6 camels hair brush.

The 120 animals with good gastric flaps were then divided into 4 groups of 30 and were painted with:

1. Polyethylene glycol 300.
2. A 0.5 per cent solution of 20-methylcholanthrene in polyethylene glycol 300.
3. A 4 per cent solution of 2-acetylaminofluorene in polyethylene glycol 300.
4. No painting; 20-methylcholanthrene thread inserted beneath the gastric mucosa.

Although several of the flaps have shown apparent tumors, biopsies of the flaps show only cystic hyperplasia. The mucosa has remained remarkably intact, with no evidence of squamous metaplasia.

THE HETEROLOGOUS TRANSPLANTATION OF HUMAN TUMORS. ABRAHAM TOWBIN* (introduced by E. von Haam). (Department of Pathology, Ohio State University, Columbus, Ohio.)

It has been proposed that heterologous transplantation of human tumors to the guinea pig eye be used as the basis for differentiating benign from malignant growths. The present study is an evaluation of the clinical applicability of the proposed technic as a diagnostic measure.

The establishment of transplants in the anterior chamber evolves through three well-defined phases: first, the suspension stage during which the transplant exists in a "tissue culture" state; secondly, the nidation stage during which the transplant becomes organized; thirdly, the stage of active growth.

Of 100 different human malignant tumors, only 2 showed active growth after long latent periods following transplantation into the anterior chamber. Transplants of 9 human tumors persisted for periods ranging from 10 to 114 days in the stage of nidation; 89 of the transplanted tumors showed steady regression.

The results in this investigation indicate that relatively few malignant human tumors can be transplanted successfully to the anterior chamber of the eye of the guinea pig.

THE EFFECT OF THE PRESENCE OR ABSENCE OF THE MILK FACTOR AND OF CASTRATION ON MAMMARY RESPONSE TO ESTROGEN IN MALE MICE OF STRAINS OF KNOWN MAMMARY TUMOR INCIDENCE. J. J. TRENTIN* (introduced by W. U. Gardner). (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Mammary responsiveness was determined by administering a constant dose of estrogen over a 2-week period prior to sacrifice, and by measuring the maximum dimension of each of the mammary glands. Both control and treated mice were sacrificed at 9 $\pm$ 1 weeks of age. Castration, when performed, took place 1 week before the onset of estrogen treatment. Comparison between the presence or absence of the milk factor was made in high and low tumor strains appropriately foster-nursed, and in reciprocal hybrids between a high and low tumor strain. In the case of the C57 strain fostered on C3H, only the fostered mice themselves, and not their descendants, were used.

In each of three high tumor strains (C3H, CBA, and A), castration increased the mammary re-

sponse to estrogen. Of three low tumor strains (C57, JK, and N) only one (JK) showed an increased mammary response to estrogen following castration. The effect or lack of effect of castration upon mammary response to estrogen was not, however, altered by presence or absence of milk factor (C3H versus C3HF; C57 versus C57F).

The mammary response of intact mice of the various strains was not consistently correlated with mammary tumor incidence. Considering castrated mice only, the three high tumor strains (C3H, CBA, and A) showed a greater mammary response to estrogen than the three low tumor strains (C57, JK, and N). Removal or addition of the milk factor by fostering did not, however, significantly alter the mammary response to estrogen (C3H versus C3HF; C57 versus C57F).

THE QUESTION OF TISSUE LOCALIZATION OF RADIOACTIVE TRIPHENYLBROMOETHYLENE. Gray H. Twombly, Erwin F. Schoenewaldt,* and Doris Meisel.* (Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

French investigators at the Curie Institute, working with a brominated form of the synthetic estrogen, triphenylethylene, and using radioactive bromine, have found a selective concentration of radioactivity in the ovaries, adrenals, and pituitary—the latter showing an activity per milligram of over 100 times that of the blood. A repetition of these observations in our laboratory has failed completely to confirm these findings. No measurable radioactivity could be found in the pituitary.

Triphenylethylene causes Leydig-cell tumor formation in male mice of the A, JK, C, and RIII strains, but not in C57 mice. Comparison of the radioactivity of the testes of A mice with C57 mice, and of RIII with dba mice, showed that this difference in response to the synthetic estrogen probably was not due to selective concentration of the estrogen in the testes of the A mice.

THE USEFULNESS OF CYTOLOGICAL STUDIES IN THE FOLLOW-UP OF CANCER PATIENTS. E. von Haam and D. Eshbaugh.* (Department of Pathology, Ohio State University, Columbus, Ohio.)

For the first 5 years, patients with various malignant neoplasms were observed by frequent cytological studies controlled by biopsies. In some cases, the cytological examination remained positive in spite of clinical disappearance of the tumor; in others, the tumor cells disappeared locally, while the tumor progressed by invasion and metastasis. Local recurrence of the neoplasm could be recognized often several months before the recurrence became evident clinically. Changes found in the cytological smears also permitted certain conclusions with regard to the local effectiveness of radiation therapy. The fallacy of basing any prognosis upon the results of cytological studies is emphasized.

A METHOD FOR THE STUDY OF TUMOR TISSUE *IN VITRO*. N. J. Wade,* E. L. Hodapp,* and Betty C. Musacchia* (introduced by Edward A. Doisy). (Department of Biology, St. Louis University, St. Louis, Mo.)

The method used is a modification of the Cohen, Borsook, and Dubnoff procedure in which tumor and liver slices 0.3–0.4 mm. thick, weighing 5–10 mg., are incubated together in a medium to which is added the chemicals being tested. Sarcoma 37 in Swiss mice and lymphosarcoma in C3H mice are used routinely.

Of several different media, the one selected for routine use consists of 2.0 cc. Krebs-Ringer with phosphate buffer and 2.0 cc. horse serum. Tissues, and materials in 20-cc. beakers, are incubated in a Dubnoff shaker incubator for 18 hours at 35° C. in 95 per cent oxygen and 5 per cent carbon dioxide at a pH between 7.2 and 7.7.

Results are determined by histological study of non-incubated controls, incubated controls, and incubated treated tissue. Lethal concentrations of chemicals cause necrosis, or complete disintegration of all cells. Sublethal concentrations show survival inferior to incubated controls. Survival of tumor is further checked by inoculation into mice. In 45 experiments with Sarcoma 37 growth of incubated controls was about 91 per cent, and it was 78 per cent in 36 with lymphosarcoma. Survival of both liver and tumor, checked by respiration in the Warburg, in 13 experiments shows good correlation with the histological picture.

Compounds tested to date show no sharp differential effect. Of 35, 10 were more toxic to liver than sarcoma; 7, more toxic to liver or lymphosarcoma than sarcoma; 8, equally toxic to liver and tumors; 3, somewhat more toxic to tumors; 6, no effect.

PLASMA AMINO ACIDS IN ACUTE LEUKEMIA IN CHILDREN. Harry A. Waisman* and Harry G. Poncher* (introduced by Harold P. Rusch). (Department of Pediatrics, University of Illinois Medical School, Chicago, Ill.)

The possibility that the dysplastic changes in the bone marrow in patients with acute leukemia may represent an aberration in the activity of enzymes concerned with the normal cellular maturation process prompted our interest in a more critical evaluation of the role of amino acids in this disease process. Initial studies have been concerned with a comparison of the plasma level of the free amino acids in children with acute leukemia and that in normal children of the same age. The use of aminopterin has provided the opportunity of comparing the free amino acids in the plasma of the same patient before and after folic acid antagonist therapy. Additional comparisons were made in the patients who showed a hematologic as well as a bone marrow remission. An attempt has also been made to correlate the role of specific amino acids with some of the clinical observations. Plasma tyrosine levels in patients who showed skin hyperpigmentation were no different than those found in normal individuals. Several patients showed an increased level of methionine during the recovery phase of the disease. Phenylalinine levels appeared to be increased after remission was induced by antagonist therapy. The possible significance of other amino acids will be presented.

CERTAIN ABNORMALITIES OF BLOOD COAGULATION OF PATIENTS WITH CANCER. JEROME M. WALDRON,* BARKLEY BEIDELMAN,* and GARFIELD G. DUNCAN* (introduced by K. E. Paschkis). (Dept. of Physiology, Jefferson Medical College, Philadelphia, and Division of Medicine, Pennsylvania Hospital, Philadelphia, Pa.)

Previous studies have shown that the oral ingestion of various fats, including cream from cow's milk, produces a sharp decrease in whole blood coagulation time. This hypercoagulability is detectable within ½ hour after drinking the cream, and in 1 hour a maximum decrease in coagulation time averaging 45 per cent is attained. Dilution of the blood with 0.85 per cent sodium chloride solution produces results typical of normal blood with the fasting sample and typical of hypercoagulable blood with the sample obtained 1 hour after feeding the cream. When the fasting blood sample is diluted with 10 per cent cream in physiological saline, the coagulation time is decreased even on extreme dilution due to the clot-accelerating properties of the cream.

These characteristic responses are obtained not only in normal healthy individuals but in all groups of diseases encountered in a general hospital with one exception: This exception is in patients with cancer. The response of patients with cancer to the oral ingestion of cream varies from a prolongation of coagulation time, which has not been observed with any other disease, to a general minimal response which on the average does not differ appreciably from the normal variations in blood coagulation time obtained by repeated venipuncture. Dilution of this blood with cream in physiological saline does not result in a normal curve. This altered response of patients with cancer to the *in vivo* and *in vitro* clot-accelerating properties of cream is independent of the type or site of cancer in the cases studied. There is no correlation with the state of nutrition, sedimentation rate, or total plasma protein.

THE ACTION OF SPLEEN EXTRACT IN MALIGNANCY. GEORGE F. WATSON. (Kitchener, Ontario, Canada.)

Administration of spleen extract to mice bearing the Balogh sarcoma consistently resulted in regression of the tumor. In later studies with Sarcomas 37 and 180 and methylcholanthrene-induced sarcoma, necrosis and regression of tumors was observed.

The extract has been given intravenously to human beings since 1934, with impressive results in various types of malignancy. Chronically ill patients have improved subjectively and objectively, and have shown remarkable gain in weight. Tumors have diminished in size or have disappeared, and some patients have survived by years their life expectancy. In patients with advanced cancer results have been less satisfactory, but even these have shown improvement. Brief summaries of the following cases diagnosed by biopsy or surgical specimens will be given: two cases of mammary carcinoma, one case of uterine carcinoma, one case of carcinoma of the tonsil, four cases of skin cancer, one case of cancer of the buccal mucosa, one case of fibrosarcoma of the forearm, two cases of carcinoma of the stomach, and three cases of rectal carcinoma.

THE CONTRASTING COLOR REACTIONS OF MALIGNANT TUMORS. EMIL WEISS. (Department of Pathology, Peoples Hospital, Chicago, Ill.)

The colors of some dyes can be reduced in contact with tissues. Malignant tumors reduce more rapidly than normal tissues or benign tumors. Small particles of tissue and vigorous shaking considerably hasten the process of reduction. Some of the reducible dyes are only slightly affected by

normal tissues or benign tumors, while malignant tumors cause a very rapidly progressing decolorization or highly contrasting changes of the original color. The dyes, with the exception of litmus, are used in dilutions of 1:10.000, dissolved in 35 per cent isoprophyl alcohol. Benzo blue, chlorazol black E., naphthogene blue 2B, sodium 2,6-dichlorobenzenoneindophenol, sodium 2,6-dichlorobenzenone indo-3′-chlorophenol are completely decolorized by malignant tumors, while normal tissues and benign tumors retain the original color, only slightly lighter. Cotton blue, litmus, and Poirrier's blue are changed by malignant tumors to a light rose color from the original blue. Trypan blue is changed by malignant tumors from the original violet to a pale blue. Tissues in amounts of $\frac{1}{4}$–$\frac{1}{2}$ cc., cut up in small particles, suffice. The tissues are placed in tubes, covered with 5 cc. of the dye, and corked. They are shaken vigorously for 1–2 minutes, and then the color changes are noted. Tubes containing malignant tumors have a turbid supernatant fluid, while normal tissues and benign tumors have a clear supernatant fluid. Malignant tumors of any type give turbidity and contrasting color reactions. The contrasting color reactions may be considered as one of the essential characteristics of malignant tumors.

THE EFFECT OF IRRADIATION ON AZO DYE NEOPLASIA IN THE RAT LIVER. I. INFLUENCE OF X-RAY AND RADIOGOLD ON THE INCIDENCE OF HEPATOMA AND THE HISTOLOGY OF THE LIVER IN RATS FED DIMETHYLAMINOAZOBENZENE. George Z. Williams, Nelson F. Young, and Jeffries P. Moore.* (Samis Grotto Cancer Research Laboratory, Department of Oncology, Medical College of Virginia, Richmond, Va.)

Sprague-Dawley albino rats were maintained 24 weeks on a synthetic diet containing 0.04 per cent *m′*-methyl-dimethylaminoazobenzene. The animals were divided into groups for adjunct treatment with 1,600–3,600 r, 200 kv, or with intrasplenic injection of 0.6 to 2.0 mc. radioactive gold colloid in single doses.

Controls fed the basal diet and the azo dye only yielded nine tumors in 38 rats (24 per cent), which survived 6 months or longer. The diet plus *m′*meDMB and 200 r x-ray 3 times weekly for 6 weeks, with a total dose of 3,600 r, resulted in 20 liver tumors in 37 animals (55 per cent); 200 r x-ray 3 times per week for only 3 weeks (total of 1,600 r), followed by intrasplenic injection of radioactive gold colloid the fourth week of azo dye diet, caused 10 tumors in 15 rats which survived 6 months or longer (66 per cent). Gross "tumors" found to be benign on microscopic examination are not included.

In animals receiving x-ray or radiogold, the tumors were larger, occurred earlier, and were multicentric. Bizarre cytologic pictures and severe damage such as vascular thrombosis and infarction occurred only in the irradiated animals. An unusual cytologic pattern found in many irradiated livers is described. The Chalkley volume-surface ratio determination revealed the following nuclear cytoplasmic ratios in the various cellular areas: hypertrophic liver cell areas, 11.8; small-cell regenerative areas, 2.0; areas of histologically malignant tumor cells, 4.4; and average for all nontumorous liver cell areas, 9.3.

It is concluded that x-ray or radiogold (chiefly beta) irradiation produces a nonspecific liver cell damage which increases the incidence of tumors produced by *m′*-methyl-dimethylaminoazobenzene.

OXIDATIVE PHOSPHORYLATION IN TUMORS. H. G. Williams-Ashman* and A. L. Lehninger* (introduced by C. G. Huggins). (Nathan Goldblatt Memorial Hospital for Neoplastic Diseases, University of Chicago, Chicago, Ill.)

A study has been made of the coupled phosphorylation of adenine nucleotide accompanying oxidations catalyzed by homogenates and cytoplasmic fractions derived from transplantable rat and mouse tumors (Jensen sarcoma, Flexner-Jobling carcinoma, a mouse amelanotic melanoma, and the Ehrlich ascites tumor).

When hexose diphosphate is glycolyzed by fortified, unfractionated tumor homogenates in the presence of fluoride, a marked uptake of inorganic phosphate occurs when pyruvate is the electron acceptor or when oxygen is the ultimate acceptor via the cytochrome system.

Washed tumor mitochondria catalyze an uptake of inorganic phosphate when succinate is oxidized in the presence of a suspension medium containing glycyl-glycine buffer, Mg ions, fluoride, adenine nucleotide, inorganic phosphate and cytochrome c. When yeast hexokinase and fructose are employed as a terminal phosphate acceptor system, the P/O ratio for succinate oxidation is greater than unity, and the phosphorylation is uncoupled by low concentrations of 2:4 dinitrophenol, as are phosphorylations in mitochondrial systems derived from normal organs. Changes in the level of inorganic phosphate associated with the oxidation of

dihydrodiphosphopyridine nucleotide will be discussed.

The presence of large, growing transplantable tumors in rats does not impair the ability of mitochondria isolated from the livers of such animals to esterify inorganic phosphate concomitantly with the oxidation of either glutamate or succinate.

HEPATOMAS IN MICE FED A SYNTHETIC DIET LOW IN PROTEIN AND DEFICIENT IN CHOLINE.

J. Walter Wilson. (Department of Biology, Brown University, Providence, R.I.)

Hepatomas have been found in two mice that had been fed from weaning for 231 days and 463 days, respectively, on the following diet low in protein and deficient in choline: casein, 4 per cent; salts, 4 per cent; corn starch, 86.5 per cent; cod liver oil, 2 per cent; Wesson oil, 3 per cent; cystine, 0.5 per cent; with water-soluble vitamins, pyridoxine, thiamine, niacin, calcium pantothenate and riboflavin incorporated into the diet (Lowry *et al., U.S. Public Health Report*, **56**:2216, 1941). The experiments were conducted for a different purpose; hence, only a very few animals were kept for this prolonged period on the diet. The mice were from our own strain in which no spontaneous hepatomas have been found. The livers became very fatty and contained considerable ceroid. Unlike those of the rats of Copeland and Salmon (Am. J. Path., **22**:1059, 1946), these livers did not develop "hobnail" cirrhosis. There was some fibrosis but no nodular hyperplasia. The tumors appeared, in the 463-day animal, as three small, smooth, rounded nodules, widely separated on the surface of the liver, and in the 231-day animal as a single protuberance. None were pedunculated or separated from the liver. In section they appear like masses of liver parenchyma without normal lobular pattern and without portal canals. They are uniformly rounded, with a compression area in the surrounding tissue. The relation of these tumors to hyperplastic nodules will be discussed.

1,2,5,6-DIBENZANTHRACENE-3,4-QUINONE AND OTHER UNIDENTIFIED QUINONES AS METABOLITES OF 1, 2, 5, 6-DIBENZANTHRACENE-9, 10-C^{14}.

George Wolf* and Charles Heidelberger. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Because quinones have been obtained as metabolites of 3,4-benzpyrene, a search was undertaken for this class of compound among the metabolites of 1,2,5,6-dibenzanthracene-9,10-C^{14}. By means of a technic involving chromatography on filter paper impregnated with formamide and solvent mixtures such as benzene-butanol or ligroin-butanol, in conjunction with chromatography on alumina, a radioactive fraction was obtained from feces of mice treated with the labeled carcinogen. This fraction, an appreciable portion of the neutral metabolites, was shown by tracer technics and solvent partitions to be reduced by zinc dust and alkali to a hydroquinone, which could subsequently be re-oxidized. Since it seemed possible that the metabolite might be dibenzanthracene-3,4-quinone, 9,10-quinone, or 3,4,7,8-quinone, these compounds were synthesized for use as carriers for purposes of identification. Because of their remarkably low solubility in cold solvents, which permits extensive washings of the crystallized fractions, purification was simplified. In carrier experiments on radioactive fractions, only the 3,4-quinone maintained radioactivity, which was, however, only a very small percentage of the total neutral metabolites. The carrier maintained constant specific activity during four recrystallizations, extensive washings, vacuum sublimation, and chromatography on silica. This metabolite was demonstrated in feces, liver, and epidermis, but the bulk of quinone metabolites is in some as yet unidentified compounds.

FUNCTIONALITY OF TRANSPLANTABLE MOUSE THYROID TUMORS.

S. H. Wollman,* H. P. Morris, and Celia Dubnik Green. (National Cancer Institute, Bethesda, Md.)

Four lines of thyroid tumors have been tested by the use of I^{131} for their ability to function as thyroid tissue. These tumors were produced by Morris and Green (in press) following prolonged administration of thiouracil to C3H mice and transplantation of the hyperplastic thyroids for several generations to new hosts (of the same strain) that also received thiouracil.

Each tumor line differs significantly from the others in its ability to concentrate iodide and to incorporate iodide into thyroxine. These lines vary in their ability to concentrate iodide all the way from being essentially nonfunctional to concentrating approximately half as well as the thyroid in the same host. The most active tumor line depresses the normal activity of the host thyroid. It appears that some tumor lines have retained their ability to convert picked-up iodide into thyroxine to a much greater extent than their ability to concentrate iodide.

THE RELATION OF THE ACID GLYCEROPHOSPHATASE ACTIVITY OF THE SERUM TO THE TYPE AND EXTENT OF PROSTATIC CARCINOMA.

HELEN QUINCY WOODARD. (Memorial Hospital and Sloan-Kettering Institute, New York, N.Y.)

Determinations of the acid glycerophosphatase in various types of prostatic tissue show that the activity of this enzyme in prostatic cancer averages somewhat less than in the normal or hypertrophied gland. In a few prostatic cancers the acid phosphatase activity is so low that the serum level is not raised, even when the disease is widely disseminated. In the majority of cases of prostatic cancer the elevation in serum acid phosphatase bears a close relation to the degree of dissemination of the disease. In the author's series, elevations in serum acid glycerophosphatase were rarely found when the disease was confined to the gland, but occurred in one-third of the cases in which local invasion had taken place and in about 70 per cent of the cases in which there were metastases iether to distant soft parts, or to bones, or to both.

CORTISONE, RELATED STEROIDS, AND TRANSPLANTED TUMORS OF THE MOUSE.

GEORGE W. WOOLLEY. (Division of Steroid Biology, Sloan-Kettering Institute, New York, N.Y.)

It has been observed that four cortical steroids, cortisone, compound F, compound A, and corticosterone, are especially active in reducing the rate of lymphoid tumor growth in the mouse if used with one or two injections per day and in suitable amounts. Oral administration of cortisone is not as efficient as subcutaneous injection of a saline suspension when a dose of 1 mg/day is used in two divided doses. The activity of cortisone and certain less active related steroids, such as 17-(α)-hydroxy-21-acetoxypregnane-3,11,20-trione, is greatly increased by multiple (3-hour) injection or feeding.

THE EFFECTS OF IRRADIATION ON AZO DYE NEOPLASIA IN THE RAT LIVER. II. CATALASE, ALKALINE PHOSPHATASE, ARGINASE, AND RIBOFLAVIN LEVELS OF THE LIVER FOLLOWING X-RAY AND RADIOGOLD IRRADIATION OF RATS FED DIMETHYLAMINOAZOBENZENE.

NELSON F. YOUNG and GEORGE Z. WILLIAMS. (Samis Grotto Cancer Research Laboratory, Department of Oncology, Medical College of Virginia, Richmond, Va.)

Sprague-Dawley albino rats were maintained for 24 weeks on a synthetic diet containing 0.04 per cent *m'*-methyl-dimethylaminoazobenzene.

Groups were treated with (*a*) 250 r (x-ray) at 250 kv 3 times weekly to 3,600 r, (*b*) intrasplenic injections of 2.0 mc. or 0.6 mc. radioactive gold colloid, and (*c*) combination of 1,600 r x-ray and 2.0 mc. of intrasplenic radioactive gold colloid (total of 168 animals).

Levels of alkaline phosphatase, catalase, arginase, and riboflavin were determined on homogenates of liver tissue. Alkaline phosphatase ranged between 40 and 100 Bodansky units with diet and dye only. In livers containing tumors but without radiation, it ranged from 75 to 110 (1 at 378), and in the livers containing tumors and subjected to irradiation, levels were from 75 to 180 with 3 above 325.

Catalase was low during periods on the riboflavin-deficient diet but rose to normal levels after the animals were returned to stock diet. Irradiation produced no significant changes, and the levels were generally higher in the livers containing tumors. The riboflavin content of the livers was low (10–12 μg. per gram) with basal riboflavin-deficient diet and azo dye, and returned to normal levels of 20 to 30 μg. on stock diet. The majority of livers of irradiated and tumor-bearing animals contained less riboflavin than normal-appearing livers without irradiation. Irradiation produced no changes in arginase levels.

It is concluded that ultimate dietary changes in catalase and riboflavin levels are not influenced by irradiation in the doses applied, and the possible significance of the changes in alkaline phosphatase is discussed. Studies are being continued to determine possible transient effects immediately following irradiation.

STUDIES ON METASTASIS. INCREASE IN NUMBER OF METASTASES WITH TIME DUE TO SUCCESSIVE RELEASES OF EMBOLI FROM A PRIMARY TUMOR.

IRVING ZEIDMAN. (Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pa.)

As recently reported, the number of metastases increases the longer a primary cancer is present in the host. Is this increase with time the result of successive releases of embolic cells, or is it due to the escape of tumor emboli in a single shower? To test the hypothesis that emboli escape at different times, experiments were planned, using transplants of Fibrosarcoma 241 in C57 black mice. This tumor regularly produces spontaneous pulmonary metastasis. Subcutaneous implants were

removed at intervals, and the mice were sacrificed several weeks later. Of mice that had borne primary tumors for 11–12 days, about 50 per cent were found to have pulmonary metastases; therefore, 11–12 days was assumed to be the time of initial release of emboli. In subsequent experiments, the implants were removed 11–12 days after inoculation in one series of mice, and after 16 days in another series. All mice were sacrificed 32 days after inoculation. Significantly more metastases were found in those mice that had borne the tumor for the longer time. It is therefore concluded that the increasing number of metastases with time is dependent upon successive releases of emboli rather than upon a single shower of emboli from the primary tumor.

AUTHOR INDEX

[When more than one abstract appears per author per page, the number is indicated by figure in parentheses.]

Announcement

Data Requested on Transplantable and Transmissible Animal Tumors

As an aid to workers in the field of cancer research, the Subcommittee on Oncology of the Committee on Pathology of the National Research Council is gathering information on the various kinds of transplantable and transmissible animal (vertebrate) tumors.

It is proposed to make available an annual or biannual list of these tumors, stating where they are currently maintained. Copies of this list will be available to workers in the field of cancer research.

An outline to facilitate collection of this material was sent out early in January to a number of research institutions and individual workers. In the first 6 weeks of our survey, 107 experimental animal tumors have been listed.

It is likely that we have not yet reached all those who are currently maintaining transplantable tumors in their laboratories. We will be glad to send copies of the outline (one for each transplantable or transmissible tumor) to those who request them.

Detailed information and supplementary material on these tumors will be appreciated, as at a later time it is hoped to publish a summary of the history and biological properties and an account of the principal experiments for which the various kinds of neoplasms have proved particularly useful.

Lucia J. Dunham, M.D.
Professional Associate
Subcommittee on Oncology
Armed Forces Institute of Pathology
7th & Independence Avenue S.W.
Washington, D.C.

In Memoriam

James B. Murphy
1884–1950

In August, 1950, a few days after his 66th birthday, a cerebral hemorrhage caused the death of Doctor Murphy at Bar Harbor, on Mt. Desert Island, Maine.

For over 40 years Doctor Murphy had devoted himself to research on cancer from a basic biological point of view. His earlier studies on the role of the lymphocyte in immunity to transplantable cancer and his later work on the existence of growth-stimulating and growth-retarding substances in normal tissues were major contributions in his chosen field.

He combined unusual executive and administrative ability with his capacity for research. As a member of boards and committees he never sought official recognition or office, but he was always clear, concise, and constructive in his contributions. Even when not opposed to a proposed plan, his opinions and criticisms had the clean-cut definiteness of a well performed surgical operation.

Because he was uncompromising in his honesty and idealism, he often gave the appearance of lacking understanding. This was far from being the actual case.

He considered the search for truth an obligation of almost religious significance. He could not, therefore, tolerate those who considered it of lesser worth. Those who were privileged to know and understand him have been permanently affected by his attitude, and continue to owe him a very great and real debt.

C. C. Little, M.D

Francis Carter Wood
1869–1951

Dr. Francis Carter Wood, international authority on cancer and pioneer radiotherapist, died January 5, 1951, of coronary thrombosis at the age of 81. Dr. Wood by training and experience had a scope of knowledge and a capacity for achievement in the cancer field that would be difficult to attain in this age of specialization. Trained as a surgeon, he recognized with characteristic foresight the importance of understanding pathology, and specialized in tissue diagnosis. He organized the pathology laboratory at St. Luke's Hospital and was its director from 1910 to 1948. As a pathologist he was particularly interested in the effects of radiation on tissues and in 1921 established the radiotherapeutic department of St. Luke's Hospital and remained director until 1948, and a consultant until shortly before his death.

When Columbia University received a bequest of $2,500,000 from George Crocker to be used for research into the causes and treatment of cancer, Dr. Wood organized the Crocker Institute for Cancer Research in 1912 and became its director. Among medical men he was a pioneer in establishing the importance of animal experimentation in the cancer field. The combined knowledge and experience from surgery, radiotherapy, and experimental pathology made him an unequaled authority on cancer but humble always when confronted by the human tragedy of an incurable cancer patient. His wise counsel and discriminating judgment as to when or why to operate, apply radiation therapy, or palliation was given freely to rich and poor alike and not infrequently passed on by younger men who learned much from their contacts with him. He was always skeptical of reported cancer "cures" and merciless in his denunciation of charlatans—remarking "there are many cures for cancer; the problem is to keep the patient alive."

Dr. Wood was born in Columbus, Ohio, December 30, 1869, son of a business man, grandson of a Columbus physician, and great-grandson of Dr. Francis Boake Carter, the founder of Starling Medical College, now part of Ohio State University. He was graduated from Ohio State University in 1891, and from College of Physicians and Surgeons, Columbia University, in 1894. He studied in Berlin and Vienna until 1896, when he joined the faculty of clinical pathology of Columbia University, serving as director from 1906 to 1912.

During his distinguished career, Dr. Wood was accorded many honors. He received honorary Doctor of Science degrees from Tufts College and from Ohio State University, the French Legion d'honneur, and the Belgian Order of the Crown. He was President of the International Cancer Congress at Atlantic City in 1939, and Vice-President of the International Union contra le cancer, President of the Radiological Society of North America and of the American Association for Cancer Research. He was a friend and admirer of Mme. Curie and after the First World War played an important part in raising funds to buy radium for her.

Many readers of *Cancer Research* remember him as Editor of the *American Journal of Cancer* from 1930 to 1941, and will recall especially the piquant comments frequently appended to his abstracts. At the time of his death he was compiling an atlas on tumor pathology. From a vast number of photomicrographs taken through the years, he had selected 1,500 to illustrate the myriad forms of malignant disease, including one from his own face. He was justly proud of his equipment and skill in microphotography.

The inspiration emanating from the personality of a great man will be missed by many friends and associates here and abroad.

Wilhelmina F. Dunning